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TREMATODES OF FISHES FROM THE RED SEA. PART 5. ON THREE NEW OPECOELIDS AND ONE MESOMETRID*

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The following descriptions of trematodes represent another contribution to the knowledge of these parasites from the Red Sea. Earlier contributions by me are dated 1937, 1941, 1942, and 1948. These trematodes were collected by the author in Egypt and their study was completed at the University of Nebraska during the tenure of a Fulbright Scholarship Award.

Type material is kept at the Department of Parasitology, Faculty of Medicine, Abbasia, Cairo, Egypt.

FAMILY: OPECOELIDAE

Opecoelus adelongatus n. sp.

Figures 1–3

Forty stained and mounted specimens were available for examination. These worms were obtained from the alimentary tracts of 25 *Upenoides vittatus* locally called "Enber baladi," from Ghardaga and Koseir, and from 5 *Mulloides auriflamma* locally called "Enber" from Ghardaga.

Description: Body narrow, elongated and almost cylindrical, with more or less parallel margins, slightly converging in anterior quarter of body. Preacetabular portion quite short, distinctly narrower than remainder of body in unpressed specimens. Body rounded posteriorly. Length 1.052 to 4.114 mm. by 0.273 to 0.595 mm. in maximum breadth. Acetabulum larger than oral sucker, located about one-ninth of body length from anterior end, slightly pedunculated, being borne on short broad stalk. Acetabulum complicated, consisting of usual muscular cup, 0.147 to 0.315 mm. in diameter, and 12 processes each having an outer covering of body cuticula and core from musculature of sucker. Eight acetabular processes located peripherally and arranged in somewhat separated pairs, one pair extending from each antero- and each postero-lateral sector of acetabulum. Each pair originating from short thick common trunk, divided distally into 2 processes, gradually tapering toward their tips. Four remaining processes digitiform, located nearer sucker opening, 2 originating anterior and 2 posterior to sucker opening which they may close when interlocked. Median processes in 1 specimen 0.042 to 0.063 mm. long, peripheral processes 0.084 to 0.105 mm. Papillae sometimes retracted and difficult to observe. Cuticle smooth and unarmed.

Oral sucker anterior, subterminal and ventral, measuring 0.082 to 0.147 mm. in diameter. Prepharynx very short, pharynx muscular, well-developed, spindle-shaped, 0.105 to 0.168 mm. long by 0.063 to 0.126 mm. wide. Esophagus of medium length, intestinal forking anterior to ventral sucker. Crura simple, laterally situated, and uniting near posterior end of body to form an arc connected to outside by short tube, opening independently through sub-terminal ventral anal pore.

Testes 2, in tandem, indented, situated in middle third of body between crura. Indentations variable, sometimes one or both testes very little indented. Testes subequal, 0.147 to 0.35 mm. in diameter, separated by variable distances in different specimens, equalling width of testes

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or much less. Vesicula seminalis externa comparatively long and tortuous, measuring about one quarter total length of worm, beginning posteriorly between crura about mid-way between ventral sucker and ovary, and proceeding anteriorly, meanwhile becoming more slender. Cirrus sac indistinct, situated usually toward left side of body at level of intestinal forking.

Ovary typically quadrilobate, pretesticular, in median plane between intestinal crura, and in anterior part of middle third of body. Ovary 0.063 to 0.21 mm. in its short obliquely directed antero-posterior dimension, and 0.109 to 0.231 in its longer oblique transverse dimension. Mehlis' gland preovarian. Ovary separated from anterior testis by width of ovary or less.

Vitelline glands profusely developed, follicles small and irregularly shaped, extending from ovarian region to posterior end of body. In gonadal region follicles occurring lateral to gonads, coming more closely together medially posterior to and between testes, tending to be arranged in 4 distinct longitudinal bands, 2 on each side more or less separated by intestinal crura.

Uterus preovarian, with transverse convolutions, between crura, opening into common genital atrium anterior to ventral sucker and lateral to median plane. Eggs comparatively few, large, ovoid, light golden yellowish, operculate and with knob-like thickening at other pole. Eggs 0.05 to 0.063 mm. by 0.029 to 0.042 mm.

Excretory vesicle elongated tubular, with parallel sides, reaching anteriorly to ovary, and lying dorsal to gonads and to posterior arch of intestinal crura.

Discussion: This species closely resembles *O. elongatus* Ozaki, 1928, but differs from that species in the presence of 2 pairs of median digitiform papillae on the acetabulum and also in the shape of the peripheral papillae.

Opecoelus upenoidis n. sp.
Figure 4

Nine specimens of this species were obtained from the alimentary tracts of 6 *Upenoides vittatus* locally called "Enber baladi" from Ghardaga. This species occurred in a mixed infection with *O. adelongatus* and was first looked upon as an aberrant form of that trematode. It was thought advisable, however, to separate the 2 forms as the differences in morphology, especially the shape of the ovary and testes, which are here entire, warrant this separation. The name *O. upenoidis* is suggested for this form.

Description: Trematodes spatulate, tapering gradually towards oral sucker, rounded posteriorly, length 1.424 to 2.398 mm. by 0.131 to 0.315 mm. in maximum breadth at level of testes. Cuticle lacking scales or spines. Acetabulum larger than oral sucker, its width often exceeding its length of 0.108 to 0.158 mm. Acetabulum set on short stalk, and provided with 4 pairs of reduced peripheral papillae and 2 pairs median.

Digestive system similar to that of *O. adelongatus.* Oral sucker 0.088 to 0.106 mm. in diameter, pharynx muscular, 0.06 to 0.088 mm. long by 0.042 to 0.07 mm. broad.

Testes 2, tandem, almost entire, separated by short space, intercrural and situated in third quarter of body, slightly more posterior than in *O. adelongatus.* Testes subequal, 0.095 to 0.147 mm. in diameter. Vesicula seminalis externa long, slightly tortuous, and extending about two thirds distance between posterior border of acetabulum and anterior border of ovary. Cirrus sac and genital opening poorly developed and lateral to esophagus.

Ovary almost entire, crescentic or triangular, sometimes provided with small indistinct lobe, and situated at posterior-most part of anterior half of body. Diameter of ovary 0.063 to 0.088 mm. Vitelline follicles distributed along intestinal crura posterior to ovary and less profusely developed than in *O. adelongatus.* Eggs few, ovoid with smooth shells, measuring 0.053 to 0.063 by 0.028 by 0.046 mm.

Discussion: This species resembles closely *O. adelongatus* from which it differs in having almost entire gonads, testes situated further posterior, and vitelline follicles less profusely developed.

Opecoelus thapari n. sp.
Figure 5

The description of this species is based upon 8 stained and mounted specimens obtained from the alimentary tracts of 6 *Upenoides vittatus* locally called *"Enber baladi"* from Ghardaga.

Description: Trematodes 0.998 to 2.34 mm. in length by 0.176 to 0.318 mm. in maximum breadth near ventral sucker, sides almost parallel, and posterior end rounded. Ventral sucker larger than oral, situated at posterior border of first fifth of body, borne on short broad pedicle. Ventral sucker 0.158 to 0.189 mm. long by 0.158 to 0.265 mm. broad, breadth usually exceeding length. Processes of acetabulum, so characteristic of genus *Opecoelus* very rudimentary and in most specimens discernible with difficulty, only 8 peripheral papillae discernible and these arranged in 4 pairs, 2 antero-lateral and 2 postero-lateral.

Opening of oral sucker directed ventrally. Oral sucker 0.066 to 0.138 mm. in diameter, smaller than ventral sucker. Pharynx muscular, well-developed, partially overlapping oral sucker dorsally. Pharynx 0.044 to 0.127 mm. long by 0.04 to 0.074 mm. broad. Esophagus of medium length, divided into 2 simple intestinal branches anterior to ventral sucker. Intestinal crura situated laterally and united to form an arch at posterior end of body, discharging through a simple short canal leading to outside at posteriorly situated anal opening.

Testes 2, smooth, ovoid or spheroid, of about same size, and situated in tandem in posterior half of body in mid-plane between intestinal crura. Anterior testis situated just posterior to equator of body and measuring 0.119 to 0.223 mm. long by 0.11 to 0.167 mm. broad. Distance between testes about equalling diameter of posterior testis. Latter 0.123 to 0.254 mm. long by 0.11 to 0.154 mm. broad. Vesicula seminalis externa long, but not tortuous. Its broad posterior end lies about midway between ovary and posterior border of ventral sucker in space between crura. Gradually narrowing toward its anterior extremity it joins the small cirrus sac just anterior to intestinal bifurcation. Cirrus sac small, ovoid, at left of esophagus, opening into genital pore near posterior border of oral sucker and near left side of trematode.

Ovary spheroidal, entire, 0.062 to 0.101 mm. in diameter, pretesticular, median and slightly pre-equatorial. Mehlis' gland near and anterior to ovary. Vitelline glands well-developed, distributed between posterior end of body to posterior border of acetabulum, but vitelline fields interrupted by gonads, thus forming 4 groups. Posterior to hind testis vitelline follicles forming an uninterrupted mass mainly between intestinal crura which may be overlapped by a few follicles dorsally or ventrally. Anterior to posterior testis follicles becoming distinctly massed into 2 groups, mainly between intestinal crura with a median space free from follicles.

Uterus preovarian, occupying intercrural space, and opening into common genital atrium, lateral to esophagus. Eggs few, large, ovoid, 0.066 to 0.07 mm. by 0.035 to 0.048 mm., operculate and with slight thickening of shell at other pole.

Discussion: This species is closest to *O. adsphaericus* Manter and Van Cleave, 1951, from which it can be differentiated by its smaller size, its acetabulum with 4 pairs of papillae instead of 3, vesicula seminalis not sinuous and longer, ovary entire not trilobed, eggs large and fewer.

I have pleasure in naming this species after Professor G. S. Thapar of Lucknow, India.

Family: Mesometridae

Arthurloossia loossi n. g., n. sp.

Figures 6–7

Nineteen specimens were stained and mounted, 5 of which were fully mature and the others were in a less developed state. They were obtained from *Pseudoscarus harid,* locally called "Wariga" or "Harid" and from *Teuthis oramen,* locally called "Sigan," from Ghardaga.

Description: Mature specimens fairly large, 6.278 to 8.17 mm. in length and 1.63 to 2.15 mm. in maximum breadth, flattened dorso-ventrally with more or less parallel sides, slightly tapered anteriorly in some specimens, but in others, especially young ones, smoothly rounded. Posterior extremity rounded, but possessing a median knob-like protuberance. Cuticle provided with minute spines, especially anteriorly. Ventral sucker lacking. Oral sucker spheroid, 0.264 to 0.29 mm. long by 0.273 to 0.326 mm. broad, deeply imbedded in parenchyma of anterior body extremity, and connected to mouth by short narrow vestibule. Esophagus long, equalling about one eighth to one sixth length of worm. Thickened walls of its posterior part simulating a poorly developed pharynx, distinguishable only in young immature worms. Intestinal crura simple, broad, slightly undulating and extending only to testes.

Testes two, obliquely situated in posterior part of body, elongated, sub-equal, 1.080 to 1.554 mm. long by 0.79 to 0.925 mm. wide. Vasa efferentia uniting to form vas deferens near

junction of second and third thirds of body. Vas deferens dilated near equator of body, forming long tortuous vesicula seminalis, latter narrowing to insignificant genital atrium opening mid-ventrally near mid-esophageal level. Cirrus and cirrus sac lacking; terminal part of male duct surrounded merely by cluster of unicellular glands.

Ovary spheroid, 0.413 to 0.616 mm. in diameter, posterior to and between testes and in plane more ventral to these. Mehlis' gland well-developed, pyriform, posterior and lateral to ovary. Uterine canal passing between testes, then forming convolutions between intestinal crura and opening into simple atrium together with male duct. Uteri of mature worms filled with thin-shelled, colorless ovoid eggs. Latter with operculum at narrow pole and lacking knobs or filaments. Eggs 0.086 to 0.09 by 0.054 to 0.062 mm.

Vitelline glands well-developed, prolific and in form of large irregularly shaped follicles extending forward from anterior border of testes to bifurcation of intestinal crura, and spreading medially as well as laterally to crura, and arranging themselves roughly in 4 longitudinal rows. Common vitelline ducts passing posteriorly between testes to reach oviduct.

Excretory system composed of at least 4 major longitudinal vessels extending backward from intestinal bifurcation to anterior border of testes. Two vessels of 1 side uniting to form a narrow duct on either side of posterior end of body. These 2 main ducts uniting at posterior level of ovary to form single short duct opening to outside terminally and posteriorly through excretory pore. Major longitudinal ducts intercommunicating and receiving others from anterior extremity of body.

Discussion: To the family Mesometridae Poche, 1926, have been assigned three genera: *Mesometra* and *Centroderma* Lühe, 1901 (this genus was considered by Lühe [1901] to be a subgenus of *Mesometra*, but it was raised to generic rank by Poche [1926]) and the genus *Wardula* Poche, 1926. The present paper adds a fourth genus, named for the eminent helminthologist, the late Arthur Looss.

TABLE 1.—*Main differences between the genera of the family* MESOMETRIDAE *Poche, 1926*

	Mesometra	*Wardula*	*Centroderma*	*Arthurloossia*
Shape of body ...	discoidal and rounded	cylindroid and elongated, length 5–7 times breadth	oval, elongated (length about 3 times breadth)	elongated (length about 3 times breadth)
Oral sucker	subterminal, superficial, opening ventrally	?	subterminal, superficial, opening ventrally	deep in parenchyma, opening through vestibule to surface anteriorly
Pharynx	poorly developed, hardly visible	flask-shaped	poorly developed, hardly visible	poorly developed, hardly visible
Testes	oval, elongated, opposite	oval, broad, close together, tandem	globular, opposite	elongate, obliquely situated
Ovary	median, lobed, broader than long	median, oval entire, broader than long	median, oval, entire, broader than long	median, spheroid, entire
Vitellaria	prolific, follicles small, occupying greater part of body, extending posterior to testes	prolific, irregular and wide-meshed, net-like, branched, occupying most of body	prolific, follicles small, extending from bifurcation of ceca to testes	prolific, follicles large, irregularly shaped, extending from intestinal bifurcation to testes
Eggs	75 by 35 microns, presumably without filament	without filament	155 by 45 microns, with long filament, measuring 175 microns long	88 by 58 microns, filament and knob lacking

The genus *Arthurloossia* is nearest to *Centroderma;* the differences between it and the other genera are tabulated in table 1.

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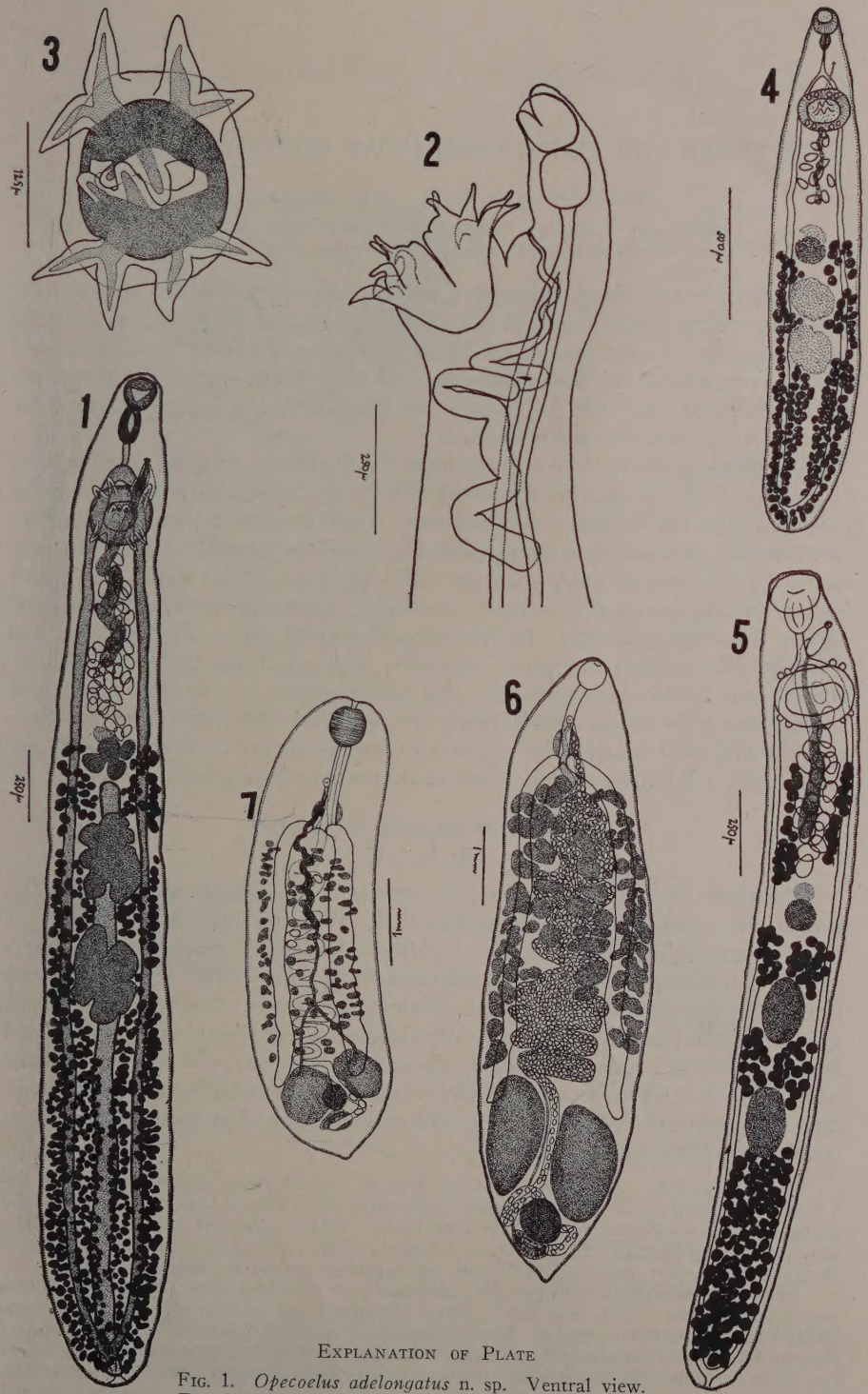

EXPLANATION OF PLATE

FIG. 1. *Opecoelus adelongatus* n. sp. Ventral view.
FIG. 2. *O. adelongatus* n. sp. Lateral view of anterior end.
FIG. 3. *O. adelongatus* n. sp. Ventral view of acetabulum.
FIG. 4. *O. upenoidis* n. sp. Ventral view.
FIG. 5. *O. thapari* n. sp. Ventral view.
FIG. 6. *Arthurloossia loossi* n. g., n. sp. Ventral view.
FIG. 7. *Arthurloossia loossi* n. g., n. sp. Dorsal view of young form.

THREE NEW FLEAS FROM JAPAN (SIPHONAPTERA)

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The flea fauna of Japan, althought a rich and interesting one, has not been exhaustively studied; and the records and descriptions are, for the most part, scattered and incomplete. As the fleas of the Holarctic Region are being more intensively studied every year, it has become apparent that many of the species and genera are quite widespread. In order to assist in the identification of Japanese fleas, three species are here described and illustrated.

The following records help to characterize the flea fauna of Japan. The majority of papers on Japanese fleas are concerned with the species that parasitize commensal rodents, and the native fauna is poorly known. The three genera discussed here indicate that there is at least one group (*Stivalius*) from the tropical Orient. Mt. Fuji now is the northernmost point where the family (Pygiopsyllidae) occurs, and it is of interest that the species in question (*S. aestivalis*) seems to occur in the adult stage only in the summer months. The presence of *Atyphloceras* (= *Saphiopsylla*) and *Rhadinopsylla* requires no special comment; both are Holarctic in distribution. *Atyphloceras* is known from one other Old World species, but this is the first record for the genus in the Orient; several species are described from North America. Several kinds of *Rhadinopsylla* are known from the Asiatic mainland near Japan, and the genus is well represented elsewhere in the northern hemisphere.

Atyphloceras shogakii, new species
Fig. 1, A–G

We follow Hopkins (1952: 365) in synonymizing *Saphiopsylla* Jordan, 1931, with *Atyphloceras* Jordan and Rothschild, 1915. With this interpretation, there is 1 species (*nupera* Jordan) in Europe, 1 (*bishopi* Jordan) in eastern North America, and 5 (*longipalpus* Stewart, *multidentatus* Fox, *felix* Jordan, *echis* Jordan and Rothschild, and *tancitari* Traub and Johnson) in western North America. The species described here is more closely allied to *nupera* and *bishopi* in the outline and chaetotaxy of the head and the labial palpus; it is, however, very different in some other respects. Our new species is widely separated, geographically and morphologically, from other named forms of *Atyphloceras*; and would perhaps be placed in a distinct genus by some workers.

Male: Head (Fig. 1, A) with 3 rows (or 4 poorly-defined rows) of preantennal setae and 4 rows postantennal setae. Clypeal tubercle considerably above antoral margin. Eye moderately pigmented, with a translucent area in the caudal part. Labial palpus five-segmented, extending approximately to the apex of the forecoxa. Antenna (Fig. 1, E) with scape bearing about 6 short marginal setae; setae of pedicel short, not extending beyond the base of the club.

Pronotal comb with 12 teeth per side; pronotum with 2 rows of setae. Mesonotum with 4 poorly-defined rows of fine setae and a caudal (marginal) row of alternating heavy and fine setae; 1 strong pseudoseta per side. Mesepisternum with about 9 setae, smaller dorsally; mesepimeron with about 12 setae, smaller dorsally. Metanotum with 3 rows of fine setae and a fourth row of heavier setae; lateral metanotal area with a heavy and a fine seta. Metepisternum with a heavy seta near the dorsal margin; metepimeron with 11 setae in three poorly-defined vertical rows, heavier caudally.

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PLATE I

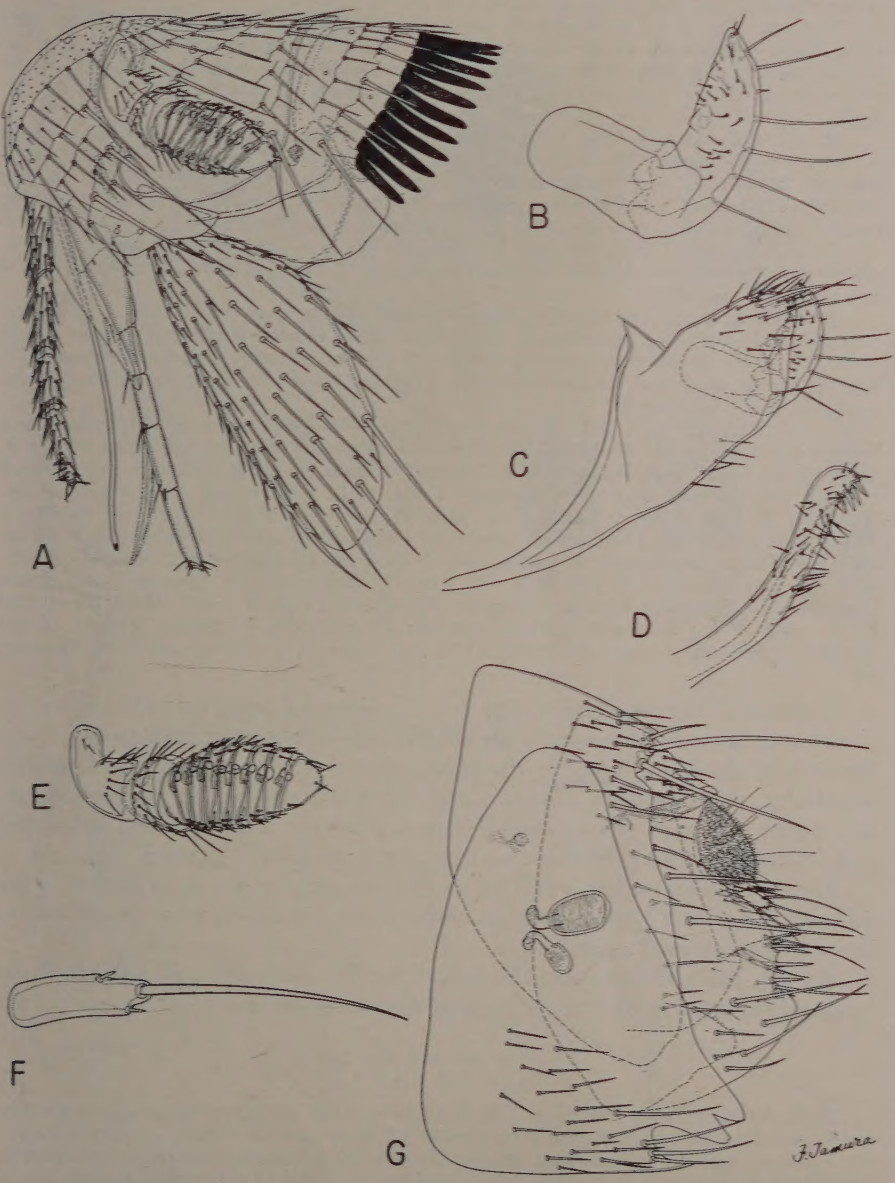

FIG. 1. *Atyphloceras shogakii*, new species: A, head, prothorax, and coxa I of male; B, moveable finger; C, clasper and moveable finger; D, sternite IX of male; E, antenna of male; F, anal stylet of female; G, terminal segments and seminal receptacles of female.

Tergum I with 3 rows of setae and a single pigmented tooth near the ventral margin. Tergum II with 2 rows of setae and 2 pigmented teeth near the ventral margin. Terga III-VI each with 2 rows of setae and a single tooth. With 3 large antepygidial bristles. Tergum VII rather expanded, and with an angulate caudal margin.

Forecoxa (Fig. 1, A) as illustrated; mesocoxa with setae anteriorly only; metacoxa with about 30 fine setae mesally on the anterior half, and with a few heavier setae laterally, near the anterior margin. Metatibia with 6 notches on the caudal margin; beginning at the base, the notches contain 2-1-2-2-2-1 setae; near the apex, on the caudal margin, are 1 small and 2 large setae.

Margin of the clasper (Fig. 1, C) entire, convex dorsally, straight and vertical caudally with setae as illustrated. Moveable finger (Fig. 1, B) apparently L-shaped, with small setae scattered laterally and 6 or 7 heavy setae on the convex caudal margin. Sternite VIII evenly rounded, with 4 heavy marginal setae. Sternite IX (Fig. 1, D) rounded and with 3 to 5 short, rounded, pigmented setae ventrally at the apex; smaller setae as illustrated.

Female: In general facies the sexes are similar. Points of difference are noted. Antenna slightly shorter than that of male. Pronotal comb with 12 teeth per side. Mesonotum with 1 or 2 pseudosetae per side.

Abdomen similar to that of male; pigmented teeth only on terga I–V. Sternite VII (Fig. 1, G) with a bisected lobe (more heavily sclerotized) below which is a rather deep sinus. Anal stylet (Fig. 1, F) about 3 times as long as wide, with setae as indicated. Stigma on tergum VIII long, typical for the genus. Seminal receptacles (Fig. 1, C) markedly dissimilar in size. Bursa copulatrix poorly sclerotized.

Types: Holotype male and allotype female from *Apodemus speciosus* (Temminck and Schlegel), Karasawa, Mt. Hodaka (elev. 8200 feet), Nagano Prefecture, 21 August 1953, coll. Tsugio Hirabayashi; deposited in the U. S. National Museum, No. 62233. Paratypes: 1 male and 1 female with the same data as the holotype; 1 male and 1 female from the type locality and type host, 4 August 1953; 1 female from *Apodemus geisha* (Thomas) from type locality, 5 August 1953, coll. Tsugio Hirabayashi; 1 male and 1 female from *A. geisha* (Thomas), 8 August 1953, coll. Tsugio Hirabayashi; 1 female from *A. speciosus* (Temminck and Schlegel), Kamikochi, Nagano Prefecture, 12 July 1952, coll. Mitosi Tokuda and E. W. Jameson, Jr.; 1 female from *A. geisha* (Thomas), Kamikochi, Nagano Prefecture, 12 July 1952, coll. Mitosi Tokuda and E. W. Jameson, Jr.; and 1 male from *A. speciosus* (Temminck and Schlegel), Hakata (Camp Hakata), Fukuoka Prefecture, 26 February 1952, coll. Major A. S. Kidwell and E. W. Jameson, Jr.

Comments: The presence of only 1 or 2 pigmented teeth per side on the abdominal segments is unique in the genus; they are numerous in most species. The female is easily distinguished by the great disparity in size between the 2 seminal receptacles. This condition is known only in *Macropsylla hercules* Rothschild, and and in such species in which a second seminal receptacle is an anomaly; and in this respect *A. shogakii* may represent a rather advanced form of the genus.

The short moveable finger and the short, stout setae on the ninth sternite of the male are also unique in the genus. *A. shogakii* does not appear to be closely related to any other species of *Atyphloceras*.

We take pleasure in naming this species for Dr. Yukio Shogaki who has made many contributions to the study of medical entomology in Japan.

Stivalius aestivalis, new species
Figs. 2, A–D and 3, A–F

Male: Head, prothorax, and coxa (Fig. 2, A) more or less typical for the genus: on the head are 4 rows of prominent preantennal setae, usually 6, 5, 4, and 3 setae to each row (from front to rear); these large setae are somewhat variable in position and there may be an extra seta between rows 3 and 4. Ten to 14 very small setae behind row 1; and 3 or 4 intermediate setae behind row 2. Three rows of postantennal setae: 5, 6, and 6 setae to each row. About 17 to 19 minute setae on dorsal margin of antennal groove. Eye well-pigmented, with a marked ventral sinus. Antennal grooves not confluent dorsally. Labial palpus five-segmented, not extending to apex of forecoxa.

Pronotum (Fig. 2, A) with 2 rows of bristles: an anterior row of about 6 and a posterior row of 6 somewhat larger bristles; ventrally the position of these bristles is variable. Pronotal

PLATE II

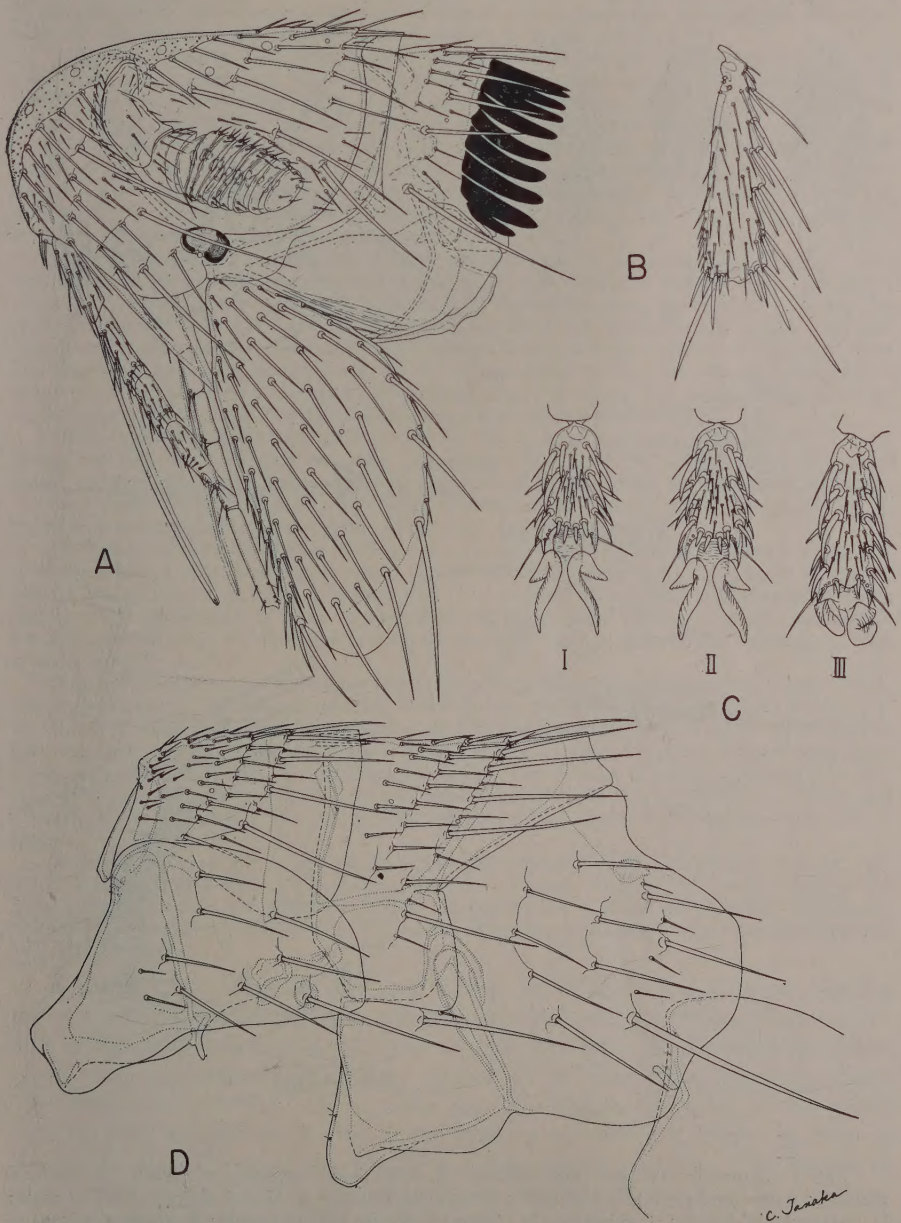

FIG. 2. *Stivalius aestivalis*, new species: A, head, prothorax, and coxa I of male; B, metatibia III of male; C, fifth tarsal segments of male; D, meso- and metathorax of male.

PLATE III

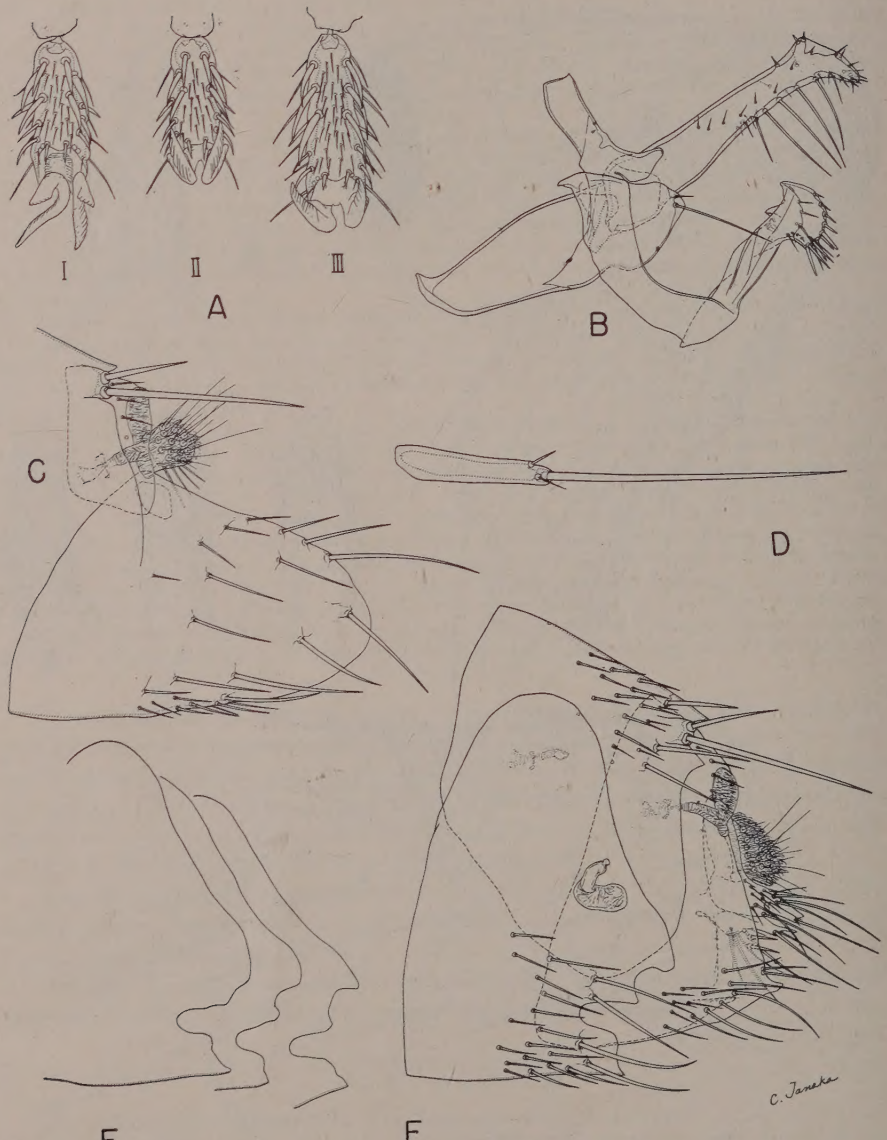

FIG. 3. *Stivalius aestivalis*, new species: A, fifth tarsal segments of female; B, clasper, moveable finger, and sternite IX of male; C. tergites VII and VIII and sternite VIII of male; D, anal stylet of female; E, variations in outline of sternite VII of female; F, terminal segments of female.

comb of about 10 spines or teeth to a side, individual teeth about as long as pronotum. Cervical sclerite hook-shaped, the ends being directed mesad. Mesonotum (Fig. 2, D) with 2 pseudosetae per side, beneath the collar; with 4 rows of setae: 5, 6, 8, and 9 setae per row, the setae increasing in size posteriorly. No apical spinelets or pseudosetae on metanotum. Mesosternum and metasternum with sclerities and setae as figured, similar to *insolli* Traub.

Abdominal terga typically with 2 rows of setae: an interior row of medium-sized setae and a posterior row of alternately large and small setae. Abdominal terga II–V each with 1 apical spinelet per side, placed rather high. With 2 antepygidial bristles, the upper slightly more than half the length of the lower. Spiracle of tergum VIII about the same size of the pygidium.

Fifth tarsal segments as illustrated (Fig. 2, C), with 6 pairs of plantar bristles, placed laterally, but not in straight lines. The stout, ventral, subapical setae of segments V are arranged 4-4-2; those on metatarsal segment V being relatively slender. Metatibia (Fig. 2, B) as illustrated: the number of setae in each dorsal notch variable according to the interpretation of the limits of the notches, but a constant character.

Modified segments as illustrated. Eighth sternum (Fig. 3, C) large, with about 10 setae near the caudal and ventral margins, and about 8 setae near the dorsal margin. Moveable finger (Fig. 3, B) about 8 times as long as wide, straight throughout most of its length, curved ventrally at its apex; with 4 or 5 slender setae along the distal half of the ventral (caudal) margin. Distal arm of sternite IX (Fig. 3, B) with a dilated apical part; laterally on this dilation there is a ridge bearing 5 or 6 slender setae, the dorsalmost being slender and the caudalmost being spiniform. On the anterior margin of this expanded part is a spur-like, sclerotized projection; on the inner surface of this expanded part is a patch of about 6 slender setae.

Female: Essentially like the male except for the terminal segments. Chaetotaxy of head, thorax and abdomen much as in the male. Anal stylet (Fig. 3, D) 4–5 times as long as wide; 2 small subapical setae, and a long apical seta, almost straight, sometimes slightly curved dorsad.

Legs similar to those of male. The stout, ventral, subapical tarsal setae (of segments V) of the male are rather slender in the female (Fig. 3, A) and arranged 2-2-2.

Outline of sternite VII (Figs. 3, E and F) variable: with a marked subventral sinus, and sometimes with a second, shallower sinus, dorsad; sometimes only the ventral sinus is present and the margin dorsad is wavy. Tergum VII (Fig. 3, F) with an acute projection both above and below the antepygidial bristles. Spermatheca slightly concave ventrally (or with the ventral margin irregular) and with a distinct concavity dorsally. Tail at right angles to the body, with a mesal crook.

Types: Holotype male and allotype female from the wood mouse, *Apodemus speciosus* (Temminck and Schlegel) from the wooded hills about 8 miles north of Kyoto City, Kyoto Prefecture; 11 June 1952. Paratypes, all from the type host, with the following data: 4 males and 6 females with the same data as the types; 2 males from the type locality, 1 August 1952; 1 male from Kyoto City, 23 August 1952; 1 male from Camp Fuji (on slope of Mt. Fuji), Shizuoka Prefecture, 28 August 1952; 5 males and 5 females from Camp Fuji, 29 August 1952; and 2 males and 2 females from Camp Fuji, 30 August 1952; specimens from Camp Fuji collected by Lt. T. C. Sicay.

Holotype, allotype, and 2 pairs of paratypes deposited at the U. S. National Museum, No. 62232. Paratypes deposited at the British Museum (Natural History) at Tring; Institute for Infectious Diseases, University of Tokyo; Entomological Laboratory, Kyushu University, Fukuoka; Department of Entomology, University of Hokkaido, Sapporo; and Canadian National Museum, Ottawa.

Remarks: This species is close to *Stivalius insolli* Traub and *S. klossi* (Jordan and Rothschild). *S. aestivalis* has about 10 pronotal teeth per side as in most members of the genus, but *insolli* has about 14 teeth per side. In *aestivalis* the apical crook of the moveable finger is short, relative to the thickness of the finger itself. The distal arm of sternite IX is rather abruptly dilated; the setae on the caudal margin of the dilation are thicker at the base in *aestivalis* and thicker apically in *insolli*. In *klossi* the apex is gradually rounded, dilated ventrally. The outline of sternite VII of the female is quite variable in *aestivalis;* 2 sinuses or a subventral sinus and a wavy margin dorsad, as illustrated. The dorsal concavity of the body of the spermatheca is distinct for *aestivalis*.

The name refers to the seasonal distribution of this species—*Stivalius aestivalius* has been collected only from June until August; this species was not found from

PLATE IV

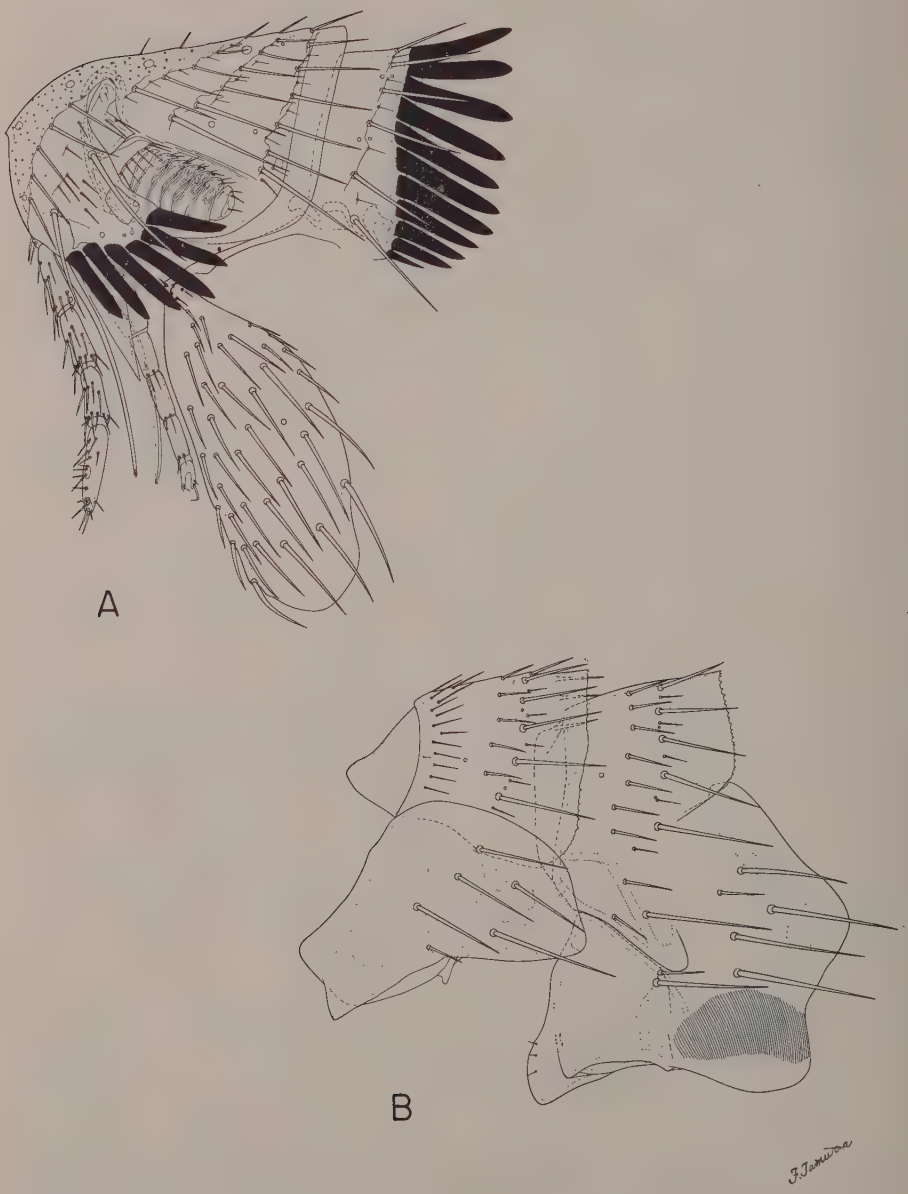

FIG. 4. *Rhadinopsylla (Rectofrontia) attenuata,* new species: A, head, prothorax, and coxa I of male; B, meso- and metathorax of male.

PLATE V

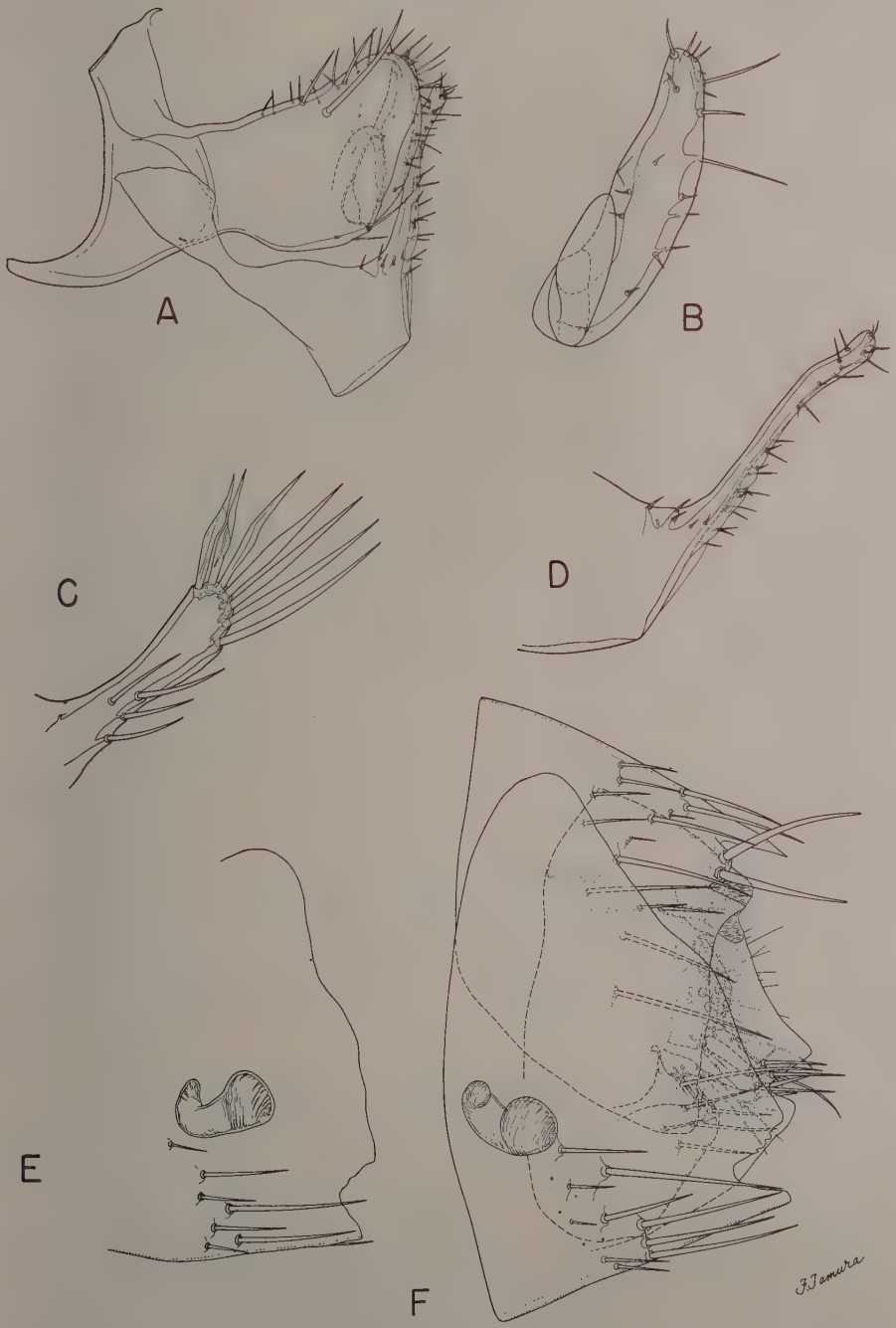

FIG. 5. *Rhadinopsylla (Rectofrontia) attenuata,* new species: A, clasper, moveable finger, and sternite IX of male; B, moveable finger; C, sternite VIII of male; D, sternite IX of male; E, sternite VII and seminal receptacle of female; F, terminal abdominal segments of female.

September through March on the type host many specimens of which were examined for external parasites.

Rhadinopsylla (*Rectofrontia*) *attenuata*, new species
Fig. 4, A–B and 5, A–F

An unnamed species of *Rhadinopsylla* is one of the less frequently found fleas on Japanese mice. The specimens are from *Clethrionomys smithii* (Thomas) and *Apodemus speciosus* (Temminck and Schlegel); all of the hosts came from the high hills or mountains.

Male: Head (Fig. 4, A) with an acute frontal angle and 6 genal teeth. There is a preantennal row of 5 moderate setae, 2 considerably longer setae posteriorly, and 6 or 7 very short setae between. Three rows of post-antennal setae: first row of 5 long setae alternating with 5 minute setae, the long setae extending beyond the bases of the setae in the second row. Eye absent. Pedicel of antenna without long setae; inner face of the club of antenna with moderately long setae on the distal half.

Pronotum (Fig. 4, A) with 11–12 heavy, pigmented spines per side. Mesonotum (Fig. 4, B) with 3 rows of setae and 3 pseudosetae per side, under the collar. Metanotum with 2 rows of setae and no pseudosetae. The sclerotic ridges and divisions of the thorax, as illustrated, are specifically constant and distinct from allied species.

Coxa III with a mesal patch of about 12–15 small, stout setae. Posterior margin of hind tibia with 6 pairs of long heavy setae, 8 smaller lateral setae and a pair of long, heavy setae anteriorly at the apex. Fifth tarsal segment of each leg with 4 pairs of lateral plantar bristles.

Each abdominal tergite with 2 rows of bristles and 2–4 pigmented, marginal teeth (4-4-4-4-3-3-2 in the holotype). Abdominal sternites with 3–5 setae ventrally.

Fixed process of clasper (Fig. 5, A) oblong, with a few delicate setae dorsally and caudally and with 1 larger lateral seta. Moveable finger extending almost to the dorsal margin of the fixed process; oblong, slightly convex posteriorly and with setae as illustrated (Fig. 5, B). Eighth sternite (Fig. 5, C) slightly expanded and with 6 or 7 flattened and expanded setae. Distal arm of the ninth sternite (Fig. 5, D) long and slender, and with small scattered setae on the caudal margin.

Female: Female similar to the male in most features. Six genal teeth as in the male. Two antepygidial bristles per side. Seventh sternite (Fig. 5, E and F) with a moderately shallow ventral sinus; for a short distance dorsad from this sinus the margin is ragged and appears broken. The base of the anal stylet is about 4 times as long as wide; there are two small subapical bristles ventrally, and a long terminal bristle. Body of seminal receptacle somewhat bulbous, the neck small and tapering.

Types: Holotype male from *Clethrionomys smithii* (Thomas), Honshu, Kyoto Prefecture, Ohara, 7 March 1952, coll. E. W. Jameson, Jr. Allotype female from the same host and locality, 3 April 1952. Paratypes as follows: 2 females with data as holotype, 7 and 8 March 1952; 1 female from *C. smithii*, Hanase, Kyoto Prefecture, 11 June 1952, coll. E. W. Jameson, Jr.; 1 female from *C. smithii*, Mt. Hiei, Shiga Prefecture, 27 March 1952, coll. Dr. Mitosi Tokuda; 1 male from *Apodemus speciosus* (Temminck and Schlegel), Mt. Fuji, Shizuoka Prefecture, 18 December 1952, coll. Lt. T. C. Sicay; and 1 female from *A. speciosus* Hata-Mura, Nagano Prefecture, 27 November 1952, coll. Tsugio Hirabayashi. Holotype, allotype and one paratype deposited in the U. S. National Museum, No. 62231. Paratypes deposited in the British Museum (at Tring) and in the collections of the authors.

Remarks: The diagnostic features of *Rhadinopsylla attenuata* are found in the head and thorax and in the genitalia. The shape of the head and thoracic segments cannot be clearly put into words, but the characters are shown in the figures. The inflated setae of sternite VIII of the male are distinct for the genus. Sternite IX of the male is similar to that of *R. tenella* Jordan, 1929, but is clearly different.

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A STUDY OF SEVERAL FACTORS INFLUENCING HATCHING OF *SCHISTOSOMA JAPONICUM* EGGS

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INTRODUCTION

Research workers have met with unexplainable and inconsistent hatching failures on the part of eggs of *Schistosoma japonicum,* even when the feces were obtained from the same individual animal and treated according to known optimum conditions. Although these inconsistencies are undoubtedly due in part to fluctuations in egg numbers, unrecognized factors probably still exist. Only a few investigators have dealt with this problem, yet it is important that the student of schistosomiasis be aware of all factors in this regard—and the diagnostician as well, since hatching can serve as a diagnostic aid.

Currently, a number of factors have been considered, including light, temperature and pH of the water, which were previously tested by Miyagawa (1916), Faust and Meleney (1924), Magath and Mathieson (1946), Stunkard (1946), and Ingalls, Hunter, McMullen and Bauman (1949). In addition, attempts were made to reach some conclusion concerning the influence which might be exerted by the design of the hatching chamber, the effect of centrifugation as a mechanical stimulus to hatching, the influence of fecal decomposition, hatchability of eggs from different hosts, and the influence of feces from varied uninfected hosts on eggs from a single animal.

METHODS

Unless otherwise designated, the eggs used were taken from bloody mucoid stools of an experimentally infected goat. The eggs were carefully washed, separated from the stool medium by the use of micropipettes and transferred to the experimental receptacles, either flask, test tube or watch glass. Unless the experiment was planned otherwise, the receptacles were maintained under optimum conditions of light, temperature and pH. Accurate counts of the eggs were made and their viability was assured by noting motility of miracidia within the shell. During each experiment, miracidia were recovered at intervals not exceeding their life-span, and accurately counted. Where necessary, contents of the incubation chamber were

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formalized at the end of the experiment and centrifuged, and the sediment examined for overlooked miracidia and unhatched eggs.

EXPERIMENTAL APPLICATION OF FACTORS AND RESULTS OBTAINED

Design of Hatching Chamber.

As noted by Ingalls *et al.* (1949) the amber colored flask with a transparent side arm, used by McMullen and Beaver (1945) for hatching and concentrating the highly phototropic miracidia of bird schistosomes, was ineffective for the less light-sensitive miracidia of *S. japonicum;* not more than 10% of the miracidia were recovered from the side arm. The hatching chamber and procedure developed by Stunkard (1946) and Ingalls *et al.* (1949) involving a flask supporting a vertical glass tube, adapted by means of a conically-bored rubber stopper, effectively concentrated the negatively geotropic miracidia of *S. japonicum.* Florence and Erlenmeyer flasks were equally effective as the hatching chamber, essentially all the miracidia being recovered from 100 eggs introduced into each type.

The Effect of Temperature on the Hatching Rate.

One hundred eggs were placed in each of a series of 30 ml. test tubes and subjected to temperatures ranging from 2° C. to 37° C. (See Table 1). Eggs hatched

TABLE 1.—*Effect of temperature*

Temperature degrees C.	No. eggs	Miracidia recovered	Eggs remaining
35–37	100	7	93
30	100	87	13
25	100	88	11
15	100	80	17
10	100	69	27
6–8	100	23	74
2–4	100	3	97

at these extremes, but at a very low rate, the optimum range being 15° to 30° C. Considerable hatching occurred at 10° C., but decreased sharply below this point. Previously Miyagawa (1916), Magath and Mathieson (1946), and Ingalls *et al.* (1949) noted 25° to 30° C. as being either favorable or optimal. Faust and Meleney (1924) established an optimum range of 8° to 37° C. Current results more nearly favor those of latter investigators. It would seem then that temperature is not a critical factor under natural conditions.

The Effect of Light on Hatching of Eggs Recovered from Six Different Hosts.

Viable eggs were obtained from a naturally infected cow, dog and man, and from an experimentally infected rabbit, goat and cat. For the latter three, eggs were recovered 70 days after exposure. The washed eggs were introduced into small dishes containing 2 ml. of water which was changed every 24 hours for a period of 5 days. One dish was placed in the light on the window sill while the other dish was placed inside a light-tight chamber. The data (Table 2) show that hatching is somewhat suppressed in darkness. Of those eggs maintained in light, 76% hatched in contrast to 48% of those in darkness, a difference of 28%, which constitutes about a 60% increase, presumably due to light. It should be noted that these findings are not in accord with those of Ingalls *et al.* (1949), who reported

TABLE 2.—*Effect of light*

Incubation period of 120 hours			Total miracidia—120 hours	
Egg source	Light or darkness	No. eggs	No.	%
Rabbit	Light	153	116	76
	Darkness	42	18	43
Goat	Light	102	80	78
	Darkness	56	26	46
Cow	Light	123	93	76
	Darkness	125	50	40
Cat	Light	211	133	63
	Darkness	210	118	56
Dog	Light	100	76	76
	Darkness	50	24	48
Man	Light	122	121	99
	Darkness	70	27	39
Totals	Light	811	619	76
	Darkness	553	263	48
Ratio	Darkness to Light 1 : 1.6			

that the rate of hatching was not significantly influenced by variations of light intensity.

Determination of the Optimum pH Range.

For this experiment, viable eggs were introduced into a series of test tubes (30 ml.) with a graduation of pH ranging from 2.0 to 8.6, prepared and tested for accuracy by using an S. Z. K. Standard Colorimeter. Below 6.8 the pH was adjusted with sodium citrate, and above 7.0 with calcium carbonate. These buffers were used because they appeared to be less toxic to the miracidia than other chemicals at our disposal.

It may be noted (Table 3) that hatching occurred at a relatively uniform rate over a pH range of 5.0 to 8.0, but was markedly suppressed above and below these levels. The results of this experiment differ from those of Ingalls *et al.* (1949), who reported that a pH below 6.6 was inhibitory and that the best results were obtained at a pH level slightly above 7.6.

The Effect of Centrifugation on Hatching.

Since centrifugation was used in some of these experiments as a method of concentrating and isolating eggs from infected stools, it was deemed advisable to determine whether centrifugation acted in any way to inhibit or increase hatching. Eggs were centrifuged at speeds up to 3000 r.p.m. for 10 minutes, then placed in the light

TABLE 3.—*Optimum pH range*

pH	Number of eggs	Remaining eggs	Miracidia recovered
2.0	100	100*	0
3.0	100	92	5
4.0	100	52	38
5.0	100	33	66
6.0	100	34	66
6.8	100	31	65
7.0	100	46	53
7.2	100	46	53
7.6	100	41	58
8.0	100	40	59
8.6	100	92	3

* Although the miracidium was capable of leaving the egg shell, it was shriveled and unable to escape from the egg shell membrane, and thus was considered unhatched.

on a window sill. Control eggs were handled similarly except for centrifugation. Miracidia were collected and counted every hour for five hours. The results indicate that centrifugation had little or no effect upon hatching.

The Effect of Decaying Fecal Matter Upon Hatching.

It has been assumed by previous workers that fecal decomposition tends to retard hatching. Five ml. of noninfected goat stool was mixed with 300 ml. of water and strained; then, 30 ml. of this strained mixture was placed in a hatching chamber and 300 viable eggs placed in this mixture. During the first 10 days, only a very small number of miracidia hatched. After 10 days, the decomposition was excessive and no further hatching was observed. This tends to confirm the consistent observations of others that great dilution of fecal debris is necessary before hatching occurs freely. The specific suppressing factor, whether products of decomposition, lack of oxygen or otherwise, has not been determined. It is interesting to speculate regarding field conditions under which eggs hatch; little or nothing is known in this regard. In addition to the presence of a stool with eggs in the vicinity of a snail habitat, coincident conditions including rainfall or irrigation must prevail to promote

TABLE 4.—*Hatchability of eggs from different hosts*

Host	Total eggs	Periodicity of hatching (hrs.)							Miracidia	
		1	6	12	24	48	72	120	Total	%
Rabbit	98	7	22	5	12	7	6	11	70	71
Goat	79	41	6	1	4	2	1	1	56	71
Cow	124	23	16	4	16	12	2	0	73	59
Cat	211	15	34	16	27	10	18	8	128	61
Dog	75	15	12	3	19	1	2	0	52	69
Man	96	16	16	5	33	5	1	0	76	79

hatching of eggs and snail exposure. The relative importance of night soil is also unknown.

A Comparison of the Hatching Rates of Eggs Recovered from Different Animal Sources.

An experiment was devised to compare the hatching rates of ova recovered from an experimentally infected goat, rabbit and cat and a naturally infected cow, dog and man. The data, recorded in Table 4, are confirmed by a previous experiment (see Table 2). The most important point of observation is that the eggs from all of these animals apparently hatched freely, and a large majority within 24 hours. Those differences which were noted might readily be explained by the differences in condition of the individual animal, and the duration and severity of the infection. The experimental exposure of the cat was heavy and the infection severe, which may have resulted in a higher percentage of immature eggs.

Comparison of Hatching Rates of Eggs Recovered From a Single Host and Exposed to the Fecal Material of Various Mammals.

Viable, well washed eggs from an experimentally infected goat, were put into an emulsion of feces from various uninfected animals. From each animal, 20 grams of feces were obtained within 24 hours after elimination and diluted with 80 ml. of water. Quantities of 2.5 ml. of this emulsion (strained) were further diluted to 100 ml. and the eggs added. A surface sample (4 ml.) was taken every hour for

15 hours (for results see Table 5). The rate of hatching in the various fecal emulsions decreased in the following sequence: horse, cow, rabbit, goat, sheep, man, dog, cat and mouse. Though naturally infected, eggs are rarely recovered from the horse. The results are relatively consistent in that the rate of hatching is higher in stools of herbivorous than in those of omnivorous and carnivorous animals. The span of hatching occurred over a longer period of time in the herbivore stool. The greater protein intake might be a significant factor in the suppression of hatching in the carnivore stool. In succeeding experiments it was shown that the eggs hatched at an increased rate and over a greater span of time when treated with calcium carbonate.

TABLE 5.—*Hatchability of eggs from a common source when exposed to feces of various mammalian hosts*

Source of feces	Total eggs	Periodicity of hatching (hrs.)							Miracidia	
		2	4	6	8	10	12	15	Total	%
Horse	205	93	37	69	13	2	2	0	176	86
Cow	435	177	66	19	13	7	12	23	317	73
Rabbit	449	104	86	31	11	8	9	0	249	56
Goat	428	99	37	32	26	13	7	1	215	50
Sheep	200	35	1	16	27	0	3	3	85	43
Man	410	72	14	0	0	1	0	0	87	21
Dog	410	74	3	3	6	3	2	3	94	23
Cat	405	41	3	0	0	0	0	6	50	12
Mouse	405	31	14	0	0	0	0	0	45	11

SUMMARY

The hatching chamber consisting of a flask (either Erlenmeyer or Florence) with superimposed glass tube (adapted by a conically bored rubber cork) was highly effective for recovery of *S. japonicum* miracidia *en masse* for experimental needs and for diagnostic purposes. Hatching occurred freely from 10–30° C, with a maximum range of 2–37° C. Although eggs hatched in total darkness, light accounted for a 60% increase. The optimal pH range was 5.0 to 8.0, the extremes being 3.0 to 8.6. Centrifugation had no effect. Fecal decomposition tends to suppress hatching. No significant difference was noted for hatchability of eggs from varied hosts. When eggs from a single animal were mixed with fecal emulsions of varied uninfected hosts, it was noted that feces of carnivores suppressed hatching.

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PARASITES OF THE GOLDEYE, *HIODON ALOSOIDES* (RAF.), IN LAKE TEXOMA

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Beginning with the summer of 1949, continuous collections of parasites have been made from the goldeye, *Hiodon alosoides* (Raf.) as a part of studies being conducted on the parasites of Lake Texoma fishes. Intensive collections were made through each of 3 succeeding summers as well as monthly collections during other seasons.

A majority of the more than 200 hosts examined were infected in the intestine with many specimens of an undescribed pseudophyllidean cestode belonging to the genus *Bothriocephalus* Rudolphi and a papillose fluke, *Crepidostomum illinoiense* Hopkins. The only other parasites we recovered from these fish were 4 immature nematodes belonging to the family CAMALLANIDAE. It is assumed that the goldeye is an abnormal host for these nematodes since only 4 immature specimens were recovered.

Bothriocephalus texomensis n. sp.
Figs. 1, 2, 3.

Specific diagnosis: Maximum length 220 mm. Maximum width 1.50 mm. Scolex of largest specimen 0.85 mm. long. Bothria deeply grooved, extending length of scolex. Terminal disc on scolex unlobed. Width of neck at junction with scolex 0.26 mm.; its length to first genital rudiment 54 mm. Width of strobila at posterior end of neck 0.80 mm. Length of youngest proglottids 0.70 mm. Mature proglottids 1.22 mm. in width by 1.00 mm. in length.

Ovary bilobed, each lobe 0.20 mm. by 0.10 mm. Oötype almost spherical, about 0.10 mm. in diameter. Vitellaria composed of 400 to 450 follicles, each follicle about 39 microns in diameter. Testes, 79 microns in greatest diameter, numbering 50 to 60 in two lateral fields, each field lying on both sides of lateral nerve cord; greater number median to nerve cord. Cirrus sac large, thin-walled with maximum length of 0.25 mm. Cirrus never observed to protrude. Vagina opening to outside close behind cirrus opening. Uterine duct confined to one side, alternating irregularly. Uterine sac spherical, its maximum diameter one-third that of proglottid. Uterine pore near anterior margin of proglottid. Genital opening median, just anterior to midlength of proglottid. Longitudinal muscular fibers present, but not in bundles. Two main lateral nerve cords and 2 principal lateral excretory canals present. Secondary excretory canals present, but number not consistent from one segment to the other. Calcareous bodies absent. Each original proglottid subdividing into 8 subsegments.

Host: Hiodon alosoides (Raf.).

Organ: Intestine.

Locality: Lake Texoma, Oklahoma.

Types: Type specimen, U. S. Nat. Mus. Helm. Coll. No. 49412, paratypes in Parasitology Coll., Univ. of Oklahoma Museum.

DISCUSSION

This worm is most closely related to *Bothriocephalus cuspidatus* Cooper, particularly as the latter is figured by Van Cleave and Meuller (1934). However, it differs from *B. cuspidatus* in several respects. It has a dominant terminal disc on the scolex which is not bilobed. The cirrus was not observed to protrude. The

ovarian isthmus is thin and almost indistinguishable. The number of vitelline follicles is 400 to 450 instead of 800 to 1000. They average 38.8 instead of 90–100 microns in diameter. The vitellaria occupy 2 distinct lateral fields and are never united either dorsally or ventrally. The uterus is seldom expanded into a sac and there is no horizontal elongation. There are always 8 instead of 32 subsegments as described by Cooper (1919).

It is recognized that there is much intra-species variation among the bothriocephalids and that one must be cautious about describing new species in the group. Only after studying hundreds of fixed and living specimens am I convinced that the parasite under consideration represents a new species. The scolex of *B. texomensis* is roughly rectangular in shape, being somewhat pointed posteriorly, and is never "arrow shaped" as described by Cooper (1917) for *B. cuspidatus*. The first segments are quite distinct from the scolex posteriorly but are never more than slightly wedge shaped. Subsegmentation occurs regularly, each original segment producing 8 subsegments as opposed to the 32 subsegments produced in each original segment in *B. cuspidatus*.

When this worm is compared to the closely related subspecies of *B. cuspidatus* established by Wardle (1932) one finds no consistent relationship between it and any one of the three subspecies. It differs from *B. c. hiodontus*, the subspecies previously reported from *Amphidon* (= *Hiodon*), in that in the latter, the apical disc is inconspicuous, the bothria shallow and the scolex is relatively short and pointed anteriorly. The scolex is somewhat like that of *B. c. luciopercae* in shape, but the two worms differ in the characters mentioned above.

Crepidostomum illinoiense Hopkins
Figs. 4 and 5

The author has enjoyed the advantage of having large numbers of both living and stained specimens of *C. illinoiense* for study, and the extreme variations noted for their general morphological features have been forcefully apparent. Because of these variations it has been difficult to arrive at a specific determination. However, these worms are identified as *C. illinoiense* on the basis of the notched condition of the median dorsal papillae and because they conform to the description of that species given by Hopkins (1934) for such important characters as the genital organs. It is easily understood, however, how there could have arisen the confusion in the taxonomy of this species mentioned by Hopkins. In studying dozens of living specimens, as well as numerous stained individuals fixed under conditions of relaxation and contraction, I have observed extreme variation in general shape and appearance. An active specimen, when dropped into a fixative or fixed under a cover glass, will contract to such a degree as to have little superficial resemblance to one removed from the host and allowed to become completely inactive before fixation. As Hopkins indicated, the variations observed by other workers probably were due to varying conditions of their specimens when fixed.

Dr. Hopkins was kind enough to loan me his entire collection of these flukes, including the cotypes used in his studies. Examination of these specimens shows that they are in a contracted condition and resemble my specimens fixed before relaxation. A careful study of Hopkins' specimens, along with my own, clearly establishes my specimens as *C. illinoiense*. Since his description was based on strongly

contracted specimens only, an emendation is given here to attain a more correct diagnosis of the species:

1. Maximum length 5.5 mm. instead of 1.2 mm.
2. Maximum width 0.48 mm. or 0.088 of the length in relaxed specimens as compared to 0.25 of length in contracted specimens.
3. Esophagus 5 times instead of 3 times the length of the pharynx.
4. Pharynx one-fourth instead of one-half the diameter of the oral sucker.
5. A distinct neck behind the oral sucker extends to the crural fork (Fig. 4). Width of neck at level of pharynx 0.19 mm. or 0.4 maximum width of body.

Relaxed specimens have been deposited in the U. S. N. M. Helm. Coll. No. 49413.

The neck region is distinctly narrower than the oral sucker and is a distinctive feature of this species. It is not lost even in highly contracted specimens. When the fluke becomes contracted, the region of the oral sucker often becomes folded under the neck to give the appearance of a much shortened anterior end (Fig. 5). As Hopkins has stated, under this condition the esophagus becomes folded and appears to be much shorter that its actual length.

All other characters are as Hopkins described them and need not be repeated here.

This record of *C. illinoiense* in *Hiodon alosoides* constitutes a new host record for the parasite, although it was reported from *Hiodon tergisus* by Pearse (1924). It has not previously been reported in Oklahoma.

SUMMARY

The goldeye (*Hiodon alosoides*) in Lake Texoma, harbors large numbers of the cestode *Bothriocephalus texomensis* n. sp. and the trematode *Crepidostomum illinoiense*.

The newly described cestode is most closely related to *B. cuspidatus* Cooper, differing from the latter in the nature of the scolex, the number, size, and arrangement of the vitellaria, the failure of the cirrus to protrude, and the smaller number of subsegments formed.

The specific diagnosis of *C. illinoiense* is expanded to describe the variations between relaxed and contracted conditions.

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Explanation of Figures

Fig. 1. Mature proglottid of *B. texomensis*. t. testes, v. vitelline follicles, and n. nerve cord.

Fig. 2. Cross section of mature proglottid of *B. texomensis*.

Fig. 3. Scolex of *B. texomensis*.

Fig. 4. *Crepidostomum illinoience*, anterior portion in relaxed condition showing distinct neck.

Fig. 5. *C. illinoiense*, anterior portion in contracted condition.

PLATE I

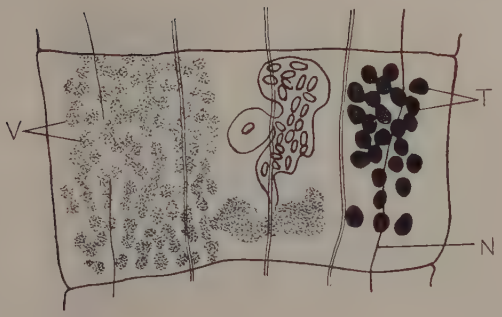

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5

THE PRESENCE OF *ENDAMOEBA HISTOLYTICA* IN THE LIVER OF GUINEA PIGS WITH EXPERIMENTAL INTESTINAL AMEBIASIS

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Within the last 4 years, the guinea pig has been found to be a satisfactory experimental host for *Endamoeba histolytica* (Carrera and Faust, 1949; Taylor, Greenberg, Highman, and Coatney, 1950). This finding has greatly facilitated studies on the pathogenicity and chemotherapy of amebiasis (Taylor, Greenberg, and Josephson, 1952; Taylor and Greenberg, 1952). The lesions produced by *E. histolytica* in the cecum and colon of the guinea pig are often very severe. However, incidental examination of the liver has shown no macroscopic lesions, and microscopic study of smears of fresh liver tissue has failed to reveal the presence of amebas even in guinea pigs that were killed in the agonal stages of amebic dysentery. Because of the great importance of the question of whether *E. histolytica* may be frequently transported to the liver from intestinal lesions, it was decided to obtain more precise information on this point. Slices of liver, approximately 1 by 1 by 0.5 cm. in size, from intracecally infected animals were inoculated into culture medium in order to detect the parasite. The cultures were seeded with organism *t*, incubated at 37° C. and examined for as much as 144 hours for growth of amebas. In addition, through the courtesy of Dr. Benjamin Highman (Laboratory of Pathology, National Institute of Arthritis and Metabolic Diseases), sections of the liver from some of the animals were examined histopathologically for evidence of damage. This preliminary report presents the data thus far obtained.

The data summarized in Table 1 show that the livers of 15 of the 20 guinea pigs of this experimental series were found infected with *Endamoeba histolytica*.[2] Seventy cultures, or 18.6 percent of 377 tubes inoculated, were found positive for amebas. In many cases the amebas were found only after incubation for 96 or 144 hours, and in some cases only in transfers from the original cultures. Cultures were reported negative only after examination several times between 72 and 144 hours. The time elapsed between the experimental inoculation and post-mortem examination of the guinea pigs is shown in Table 1. The earliest examination was performed 7 days after injection; in this case the liver was found infected, by culture, and the intestinal lesions were graded as most severe. As yet, however, the data are too meager to indicate a relationship between the severity of the intestinal lesions and the occurrence of the parasite in the liver. In a series of cases not tabulated here, there was no parasitism of the liver when the intestinal infection was detectable only by finding parasites within the lumen without demonstrable lesions in the wall. Data from the table show that the left lobe of the liver was found infected, in some cases even when the right lobe was not invaded. The proportion

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[2] All the infections were with NIH strain 200 except for one series in which the F 22 strain was used and the parasite found in the liver.

of left-lobe infections in this series of guinea pigs is higher than that reported for amebic abscesses in the left lobe of human cases.

Nine of the 20 animals were studied histopathologically; 5 of these 9 were found positive by the culture technique. The pathologist's report shows neither amebas nor damage attributable to amebas in any of these guinea pig livers. It is not improbable that these livers were infected with bacteria as well as amebas, but further work is needed for definitive information on this question. Carrera and Faust (1949) reported one incidental finding of infection of the liver with amebas, detected on culture, in a guinea pig with experimental amebic dysentery. The present paper provides confirmation of this finding and shows in addition that under such condi-

TABLE 1—*Results of examination of cultures of whole egg medium for* Endamoeba histolytica, *inoculated with pieces of liver of guinea pigs having amebic dysentery*

Number	Guinea pig		Tubes inoculated			Tubes positive for *E. histolytica*		
	Days since inoculation	Pathogenic index	Right lobe number		Left lobe number	Right lobe		Left lobe
1[1]	18	4[2]		12[3]			1[3]	
2	10	4		6			4	
3	11	4		20			11	
4	19	3		20			7	
5	15	4	10		10	4		0
6	18	3	9		10	3		0
7	19	4	10		10	4		1
8	9	4	10		10	4		0
9	13	4	10		10	1		0
10	18	4	10		10	0		0
11	15	4	10		10	0		0
12	16	4	10		10	3		2
13	18	3	10		10	0		0
14	7	4	10		10	2		3
15	8	4	10		10	0		1
16	9	4	10		10	0		2
17	9	4	10		10	2		5
18	11	4	10		10	0		0
19	13	4	10		10	5		5
20	14	4	10		10	0		0
Totals 20			159	58	160	28	23	19

[1] Died during night, autopsied several hours after death.
[2] Calculated on an arbitrary scale of 1 to 4; 3 indicates gross lesions in the cecum with demonstrable amebas in the fresh smear; 4 indicates that, in addition, there were gross lesions in the colon.
[3] No distinction made between right and left lobes.

tions passage of amebas from lesions in the intestinal tract to the liver may be of frequent occurrence. The data suggest the possibility that human intestinal amebiasis may be frequently accompanied by infection of the liver, and this information may have bearing on the elucidation of the etiology of cases of hepatitis currently considered as of unknown origin.

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COMPARATIVE SUSCEPTIBILITY OF COMMON LABORATORY ANIMALS TO EXPERIMENTAL INFECTION WITH *SCHISTOSOMA HAEMATOBIUM*[1]

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Brumpt (1928) listed the animals which at that time had been reported by various investigators to have been successfully infected with *Schistosoma haematobium*. This list included the following: various species of monkeys, guinea pigs, white mice, white rats and hedgehogs. Stunkard (1946), in a search for a suitable molluscan host for *S. haematobium* among snails native to the United States, used jerbils and baboons, which had been infected in Egypt, as a source of eggs. Watson, Abdel-Azim and Halawani (1948) used mice as experimental hosts for *S. haematobium* in carrying out chemotherapeutic studies. Standen (1949) reported the successful infection of the golden hamster with *S. haematobium*, but lacked sufficient material to make any observations on the course of the infection in the hamster. In the above reports, no mention is made of the number of cercariae used for exposure or of the resulting worm yield or other pertinent information which is of value in experimental schistosome infections.

In order to maintain experimental *S. haematobium* infections in a laboratory situated outside the endemic area, and to conduct investigations on various biological aspects of this infection, it is of the utmost importance to know which of the common laboratory animals are susceptible to this schistosome, and to determine the expected worm yield and percentage worm recovery following standardized cercarial exposures. The rate of maturation and location of the worms in the host, observations on the gross pathology and the passage of viable eggs in the feces and/or urine of the host constitute essential criteria for the determination of the most favorable laboratory animal to be used for *S. haematobium* infections.

MATERIALS AND METHODS

Cercariae for some of our animal exposures were obtained from *Bulinus truncatus* exposed in Egypt and sent to us by Dr. C. H. Barlow. Other animal exposures were made with cercariae from our own laboratory reared and infected *B. truncatus*. Our stock of snails was started through the courteous cooperation of Dr. C. H. Barlow, and the late Dr. M. Abdel-Azim, both of the Bilharzia Snail Destruction Section of the Ministry of Health, Cairo, and of Dr. Robert E. Kuntz, NAMRU No. 3, Cairo, Egypt. Snails were reared, exposed and maintained according to the method described by Moore, Thillet, Carney and Meleney (1953).

In order to obtain cercariae for animal exposures, shedding snails were placed in a crystallizing dish (50 by 30 mm.) filled about half full with spring water. The dishes were placed under a light on a dark background. We observed that the emer-

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gence of cercariae from the infected snails takes place between the hours of 11 A. M., and 3 P. M. The counting of cercariae and percutaneous exposure of the animals were done according to the methods described by Moore, Yolles and Meleney (1949) for *S. mansoni*. Worms were recovered by the perfusion technic of Yolles, Moore, DeGiusti, Ripsom and Meleney (1947). In addition to the regular perfusion of liver and mesenteries, a third area, the lower extremities including the pelvic circulation, was perfused after the mesenteric perfusion was completed. This third perfusion was accomplished by tying or clamping the cut end of the portal vein and removing the tie on the vena cava, which is severed at the time of removal of the liver. A second entrance was made in the thoracic aorta for this additional perfusion.

Fecal examinations on all animals were done by the saline sedimentation-hatching technic with a microscopic examination of the sediment prior to hatching.

RESULTS

MICE—A total of 47 albino mice, CFW strain, were each exposed percutaneously to 150 cercariae. The animals were sacrificed at two-week intervals from 6 through 14 weeks after exposure. Table 1 shows the recovery, maturity and distribution of the worms in these mice. The average worm recovery for this group of mice was 5.3 worms per mouse or 3.5% of the cercariae employed for exposure. No mature worms were recovered until 10 weeks when 44% of the worms recovered were mature. At 12 weeks 84% were mature and at 14 weeks all of the worms recovered were mature. This is in agreement with Watson, *et al.* (1949), who state that *S. haematobium* reaches maturity between 80 and 100 days. At 6 and 8 weeks after exposure, a large percentage (78% and 69% respectively) of the worms were recovered from the liver. This is in keeping with the expectation since the schistosomes generally have a tendency to remain in the liver during their maturation. At 10 weeks only 38% of the worms were recovered from the liver, but at 12 and 14 weeks, over 50% of the worms were still in the liver. The residence of more than 50% of the worms in the liver after they have all reached maturity, may be taken as an indication that the mouse is not as favorable a host as one in which the majority of mature worms are found in the mesenteric circulation.

At the time of autopsy, a careful gross inspection was made of the vesicular circulation, prior to perfusions. In one or two instances, after perfusing the lower extremities, one worm or a pair in copula were found in the perfusate, however, since no lesions were seen in the bladder and no worms were observed in the vesicular circulation prior to perfusion, we must assume that the few worms found had been trapped in the loops of intestine after being washed out of the mesenteric circulation during the perfusion. On the basis of our findings, we must conclude that in mice, there is no migration of worms from the mesenteric circulation to the vesicular circulation up to 14 weeks after exposure.

No gross pathology attributable to schistosomiasis was observed until 12 weeks after exposure, when several discrete white spots were observed on the liver in most of the mice examined. At 10 weeks the tissue press preparation of the liver of one animal out of 10 revealed a few non-viable eggs. Twelve weeks after infection all 10 animals showed viable eggs in the liver, 3 also showed eggs in the colon, 1 showed eggs in the liver and lung, and 1 showed eggs in the liver, ileum and colon. Fourteen weeks after infection, the eggs were found only in the liver, ileum and colon with the majority of animals showing eggs in the liver and colon only.

Viable eggs were first found in the feces of infected mice 15 weeks after exposure. This is in accord with the findings of Abdel-Azim and Barlow (1948), who found non-viable eggs in the feces of their infected mice as early as 12 weeks and viable eggs between 14 and 16 weeks after exposure. Egg passage in our mice was quite sporadic and eggs were never very numerous. In one instance, we found viable eggs in the feces of infected mice 62 weeks after infection.

HAMSTERS—A total of 65 hamsters were each exposed percutaneously to 200 cercariae, after clipping the hair from the posterior half of the animal. The animals were sacrificed at two-week intervals from 6 through 20 weeks after exposure with the exception of 7 animals sacrificed at 9 weeks after exposure. Table 2 shows the recovery, maturity and distribution of the worms from these animals. The average worm recovery for this group of hamsters was 33.8 worms per hamster or 16.9% of the cercariae employed for exposure. No mature worms were recovered until 9 weeks after exposure when 4% of the worms were mature. All of the worms were mature at 10 weeks. From 6 through 9 weeks a higher percentage of worms was recovered from the intra-hepatic circulation than from the mesenteric circulation. At 10 and 20 weeks, the worms were equally distributed between liver and mesentery. At 12 weeks a slightly higher percentage of worms were found in the liver, and at 14, 16 and 18 weeks, about twice as many worms were recovered from the mesentery than from the liver. This is in contrast to the findings in mice (Table 1.), and may be interpreted as one indication that hamsters are more favorable hosts than mice for *S. haematobium.*

As with the mice a careful inspection of the vesicular circulation was made at the time of autopsy, and this circulation was perfused after the perfusion of the mesenteric circulation. This area is not included in the results shown in Table 2, because the results were not conclusive and any worms found were included with the mesenteric worms in Table 2. With the exception of the animals autopsied at 6 and 12 weeks, one or two worms were found in the perfusate of pelvic circulation, in 1 to 3 animals examined at each age level. Five pairs of worms were found in this perfusate from 1 animal autopsied 16 weeks after exposure. No eggs were observed in the bladder of this animal, and it is quite possible that these worms had been trapped in the folds of the intestine after the mesenteric perfusion There was no good correlation between the presence of eggs in the bladder, and the finding of worms in the perfusate from the pelvic circulation. The question of the migration of the worms from the mesenteric to the pelvic circulation in hamsters needs further careful consideration.

No gross pathology was observed in the animals until 12 weeks after exposure. At this time, discrete grayish-white areas were observed on the liver and the surface of the liver presented a roughened appearance. In 1 animal 2 amber colored raised areas about 1 mm. in diameter were observed on the lung. These lesions each contained a single egg and are indistinguishable from the lung lesions observed in *S. mansoni* infections, (Moore, *et al.* 1949). Eggs were first observed in the tissue at 10 weeks when they were found in the liver of 5 out of 10 animals examined. One of these 10 animals also showed eggs in the colon. At 12 weeks all 10 animals showed eggs in the liver, 2 had eggs in the colon, and 1 had eggs in the lung. At 14 weeks all 7 animals showed eggs in the liver; 4 had eggs in the colon; 3 had eggs in the ileum and one also had eggs in the lung and bladder. From 16 weeks through

TABLE 1.—*Recovery, maturity and distribution of* S. haematobium *in mice following percutaneous infection*

Duration of infection in weeks	Number cercariae per mouse	Number of mice	Total cercariae used	Number of worms recovered					Maturity of worms percentage		Distribution of worms percentage	
				Total	Mature		Immature					
					liver	mes.	liver	mes.	mature	immature	liver	mes.
6	150	8	1200	51			40	11		100	78	22
8	150	10	1500	49			34	15		100	69	31
10	150	10	1500	45	1	19	16	9	44	56	38	62
12	150	10	1500	55	27	19	9		84	16	65	35
14	150	9	1350	48	27	21			100		56	44
Total		47	7050	248								

Average worms per mouse—5.3
Percentage worm recovery—3.5%

20 weeks, all animals examined showed eggs in the liver and colon, all but 4 of the animals also showed eggs in the ileum. Eggs were also found in the lungs of a few of the animals, and in one instance eggs were seen in the jejunum. As indicated above, eggs were not found in the bladder until 14 weeks after exposure, indicating that the migration of the worms from mesenteric to pelvic circulation may take place between 12 and 14 weeks. The fact that from 14 through 20 weeks only 3 animals out of a total of 26 examined showed eggs in the bladder, indicates that the migration of the worms into vesicular circulation is only sporadic, at least up to 20 weeks. In a previous study on the histopathology of experimental schistosome infections (Meleney, Sandground, Moore, Most and Carney, 1953), we found bladder lesions containing eggs in all 6 hamsters with *S. haematobium* infections from 33 to 67 weeks duration. This might be an indication that the migration of the worms from mesenteric to pelvic circulation in hamsters may be more consistent in older infections. With regard to the colonic egg lesions, the majority of the lesions were seen in the ascending and transverse colon, with few or no eggs found in the descending colon. The greatest concentration of eggs was at the hepatic flexure.

Viable eggs were first found in the feces 10 weeks after infection. The number of eggs passed by the infected hamsters increased for several weeks, and tended to decrease about 20 to 25 weeks after infection. We have observed the passage of viable eggs as long as 75 weeks after exposure.

TABLE 2.—*Recovery, maturity and distribution of* S. haematobium *in hamsters following percutaneous infection*

Duration of infection in weeks	Number cercariae per hamster	Number of hamsters	Total cercariae used	Number of worms recovered					Maturity of worms percentage		Distribution of worms percentage	
				Total	Mature		Immature					
					liver	mes.	liver	mes.	mature	immature	liver	mes.
6	200	6	1200	242			209	33		100	86	14
8	200	6	1200	234			138	96		100	59	41
9	200	7	1400	157	2	5	102	48	4	96	66	34
10	200	10	2000	418	210	206		2	99.5	0.5	50	50
12	200	10	2000	310	184	126			100		59	41
14	200	7	1400	203	67	136			100		33	67
16	200	6	1200	215	71	143			100		33	67
18	200	7	1400	257	80	177			100		31	69
20	200	6	1200	162	81	81			100		50	50
Total		65	13,000	2,198								

Average worms per hamster—33.8
Percentage worm recovery—16.9%

ALBINO RATS—A total of 5 albino rats was exposed percutaneously to 500 cercariae each after clipping the hair from the posterior half of the animal. The animals were autopsied from 6 through 14 weeks after exposure. Only 2 animals were found to be infected, and the worm yield was 1 and 2 immature worms respectively. The average worm recovery for the group was 0.6 worms per animal or 0.12% worm recovery.

GUINEA PIGS—A total of 14 guinea pigs was exposed percutaneously to 500 cercariae each after clipping the hair from the posterior half of the animal. The animals were autopsied at two-week intervals from 6 through 14 weeks after exposure. Only 2 of the animals were found infected. One immature worm was recovered from 1 animal, 10 weeks after exposure, and 10 immature worms were recovered from 1 animal autopsied 12 weeks after exposure. The average worm recovery for this series was 0.78 worms per animal or 0.16% of the cercariae employed for exposure.

RABBITS—A total of 7 rabbits was exposed by ear immersion to 1,500–11,000 cercariae per animal. The animals were sacrificed from 17 through 23 weeks after exposure. No worms were recovered from any of these rabbits.

SUMMARY AND CONCLUSIONS

The most satisfactory experimental animal for schistosome infections is one in which the worms are able to attain sexual maturity and deposit eggs in satisfactory locations so that they may be excreted in the feces. It is important to know the prepatent period of the infection and the expected worm recovery after the exposure of the animal to known numbers of cercariae.

In this study, albino mice, Syrian hamsters, albino rats, guinea pigs and rabbits were used. Only the mice and hamsters were found to be susceptible to infection with *S. haematobium*. Using 47 mice exposed percutaneously to 150 cercariae each, a recovery of 5.3 worms per mouse was obtained or 3.5% of the cercariae employed for exposure. In the 65 hamsters exposed percutaneously to 200 cercariae each, a recovery of 33.8 worms per hamster was obtained or 16.9% of the cercariae employed for exposure. Albino rats, guinea pigs and rabbits proved to be either completely or nearly completely refractory to infection with *S. haematobium*.

In the mice, the worms reached sexual maturity between 12 and 14 weeks after exposure, but more than 50% of the mature worms were found in the liver. In the hamsters, all of the worms reached maturity at 10 weeks after exposure, and a greater proportion of mature worms migrated to the mesenteric veins where eggs which were deposited had an opportunity to be excreted in the feces. In both mice and hamsters, intestinal egg lesions were essentially limited to the ileum and colon. In hamsters, the colonic egg lesions were most numerous in the ascending and transverse colon, with the greatest concentration of eggs found in the hepatic flexure. Few or no eggs were seen in the descending colon.

No bladder lesions were seen in any of the mice, indicating that there is no migration of worms from mesenteric to vesicular circulation up to 14 weeks in mice. Only a few hamsters exhibited bladder lesions, the earliest being observed at 14 weeks after exposure, indicating that the migration of worms to the pelvic circulation in hamsters is not consistent, at least up to 20 weeks.

Viable eggs were first found in the feces of infected mice 15 weeks after exposure. In hamsters viable eggs first appeared in the feces at 10 weeks. Egg passage in the mice seemed to be sporadic, while in the hamster the number of eggs passed increased for several weeks, and began to decline about 20 to 25 weeks after infection. Viable eggs were found in the feces of mice 62 weeks after infection and in hamsters 75 weeks after infection.

Considering the factors of worm recovery, prepatent period, migration of the mature worms to the mesenteric circulation, and passage of eggs in the feces, the hamster is the most suitable animal of those employed in these experiments.

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A NEW MYXOSPORIDIAN, *MYXOSOMA SQUAMALIS*, PARASITE OF SOME SALMONOID FISHES[1]

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INTRODUCTION

For several years two commercial trout farms located near Seattle and one at Olympia, Washington experienced a considerable loss of rainbow trout (*Salmo gairdneri* Richardson) due to a disease or diseases. Diseased fish examined in 1951 exhibited many tiny, milky-white pustules on their bodies (Figure 1.)

On occasion adult silver salmon (*Oncorhynchus kisutch* Walbaum) and chum salmon (*Oncorhynchus keta* Walbaum) show similar whitish pustules on the body surfaces. Infected silver salmon are apparently canned without removal of the in-

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tegument, but the pustules are not known to cause a reduction in the quality of the pack. The unappetizing appearance, however, may lower the value of this species when sold on the fresh-fish market.

Many species of myxosporidians have been reported from the integument of fishes. These differ from the present species in spore characteristics and usually in the specific site of infection. Two that possess the iodinophilous vacuole and differ in other spore characteristics, but have the ability to break down the scale substance have been reported.

Chakravarty (1939), working on *Myxobolus mrigalae* Charkravarty, present in *Cirrhina mrigala,* reported large cysts "on the scales of fish." He observed that "the fish scales seemed perforated after taking out the cysts. They are bounded by a fibrous inner layer which was surrounded by an outer layer of cells derived from the epidermis of the host."

Keysselitz (1908) named a new species *Myxobolus squamae,* which appears in the barbel, *Barbus fluviatilis.* The site of infection was the inner surface of the scales, in which an excavation is made. He noted that the myxosporidian bodies may be 1 to 8 on a scale and are enclosed in a strongly developed connective tissue envelope.

Shaw, Simms, and Muth (1934) very briefly mentioned the occurrence of a myxosporidian parasite "in the skin" of a silver salmon presumably from Oregon. They considered the parasite to be *Myxobolus squamae* Keysselitz. Judging from the photograph of an infected fish, the appearance of the skin is similar to that of fishes infected by the species here reported.

The site of infection of the sporulating trophozoite in the present species is within the scales; this, plus the observations on spore characteristics and its selection of the rainbow trout, the silver salmon, and the chum salmon as hosts, indicate that it has not been reported in the literature.

Zenker's solution and 10 percent formalin were the fixatives used for tissue to be sectioned and stained with Delafield's haematoxylin, counter-stained with alcoholic eosin. Nearly all sections were cut between 5 and 20 microns, the majority at 15 and 20 for minimum displacement of tissues.

The hanging-drop method was not necessary for fresh spore measurements, because in the usual smear technique, scale debris, present in all preparations, supported the coverglass so that no pressure was placed on the organisms. All drawings and measurements were made with the aid of a camera lucida. All measurements of fish are given in terms of total length, defined as the distance between the tip of the snout and the most posterior portion of the caudal fin. Terminology used in this study is that used by Kudo (1920, 1946).

EFFECT ON THE HOST

The extent of infection in some cases was considerable. A 12-cm. rainbow trout was covered with infected scales, so numerous that a count was impossible, but most individuals exhibited a weaker infection. The size of some of the infected fish from trout farms ranged from 9 cm. to 16 cm. Of 1,687 silver salmon examined at two canneries in Anacortes, Washington during the fall of 1952, 86 were infected or about 5.1 percent. One silver salmon, 65 cm. in length, had 140 infected scales on the right side and 147 on the left. Only 1 chum salmon in a small number examined was found infected.

Tissue sections were made from 3 rainbow trout, 2 silver salmon, and 1 chum salmon. In 1 rainbow trout, free from external trematodes, slight changes in the epidermis above infected scales were noticeable in sectioned material. A substantial reduction in numbers of mucous cells and a thinning of epithelial cells was evident (Figure 2). Nearly all the fish from this trout farm harbored a nematode, *Cucullanus truttae,* in the pyloric caeca.

In sections from all fish the connective tissue above the scales in the scale pockets showed various modifications. The fibroblasts became much more loosely arranged and appeared to be very delicate and spindle-shaped. Tissue preparations revealed that the margins of the scales not split by the parasite were modified. Usually, the free edge rolled beneath the scale which thus had a hook-like appearance.

On some infected scales a growth was noted which covered the entire inner side of the scale and possessed many nuclei of various shapes (Figure 3). This may be a pronounced hyperactivity of the host tissue in response to the parasite. However, that this is, in fact, wholly or partially modified host tissue and no portion of it is myxosporidian is highly uncertain. Occasionally spores were noted in this location, apparently occurring naturally and not displaced to this location by the technical procedures.

All infected fish revealed that the nuclei of the muscle cells were hypertrophied. They were spherical, spheroidal, or irregular in shape, and apparently were rich in chromatin as evidenced by their affinity for nuclear stain. Similar responses by host tissue to histozoic Myxosporidia have been noted many times in the past—Hahn (1915, 1917), Kudo (1926, 1929), and Nigrelli and Smith (1938).

No evidence was found that the disease under study was the sole cause of the death of any of the fish.

Myxosoma squamalis n. sp.

Trophozoite: The sporont can be easily obtained by teasing apart infected scales which do not show a mature infection. The fresh sporont is roughly circular to oblong in outline and may be monosporoblastic or disporoblastic (Figure 4). A typical sporont measured 19 microns, and the range is from 16 to 24 microns, based on only five measurements. The cytoplasm of both fresh and Giemsa-stained sporonts revealed numerous, small vacuoles.

Regarding the arrangement of the sporonts within the tumor, the usual reports mention that they are to be found mostly in the periphery of the trophozoite, and the center is occupied by free spores. In this case, examination of sections seems to indicate that sporonts may be present anywhere in the trophozoite within the infected scale. The size of the trophozoite varies with the size of the scale.

Spore: The spore is rounded to slightly oblong in front view, pyriform in side view (Figure 5, A and B), and lenticular in end view (Figure 5 C). The sutural ridge, which is without sutural markings of any kind is narrow, the sutural line indistinct. Closely bounding either side of the sutural ridge is a narrow parallel ridge that runs completely around the valve. This is readily noted in edge view. The shell valves, devoid of appendages, are relatively thin throughout and of equal size.

The convergent pyriform polar capsules are of approximately equal size and are located at the anterior end of the spore (Figure 5, A and D). The discharged polar filaments are of equal length (Figure 5 E). Polar filaments may be readily seen

TABLE 1.—*Dimensions of fresh spores*

Character	Mean (microns)	Standard deviation	Range (microns)	Number examined
Valves				
length	9.0	0.37	8.1–9.9	110
breadth	8.6	0.36	7.7–9.9	110
thickness	6.7	0.46	5.6–7.7	110
Polar capsule				
length	4.4	0.31	3.9–5.1	110
width	3.1	0.25	2.6–3.9	110
Polar filament				
length	35.2	2.21	30.9–38.3	50

coiled in the capsule in fresh material, but only with difficulty in preserved material. Four turns or windings are noted.

Despite rather prolonged exposure (1 week) to freshly-prepared Lugol's solution, no iodinophilous vacuole was found in spore smears made of fresh or formalin-preserved material. The single, granulated sporoplasm, when stained, reveals 2 nuclei. The dimensions of fresh spores mounted in natural fluids and measured at random are analyzed in Table 1.

Spores fixed in 10 percent formalin for a period of nearly 4 years, stained in Giemsa stain, and mounted in cedarwood oil, were measured. The analyses are given in Table 2.

The mean dimensions of stained spores, when compared with those of fresh spores, showed a reduction of 18 percent in valve length, 23 percent in breadth, 16 percent in width of polar capsule, and 19 percent in length of polar capsule.

Extrusion of filaments was noted in the present species, using spores kept on ice about 24 hours after the death of the silver salmon. Extrusion was stimulated by using the digestive juices of a freshly-killed rainbow trout 16.5 cm. in length. Five polar filaments were seen in the act of everting, but the amoebula was not seen to leave the shell valves. The filaments of a single spore did not evert simultaneously. When a filament everted, the spore would recoil in an opposite direction and spin rapidly at first then slowly until it came to a stop. Only a few revolutions were actually made in the medium of pus. The application of pressure to the coverslip also caused extrusion of filaments with regularity.

The form of the spore of the *Myxosoma* under study is believed to differ sufficiently from closely related species to be considered a new species, and the name *Myxosoma squamalis* n. sp. is suggested. Material upon which the description of the organism was based is in the collection of Dr. James E. Lynch at the School of Fisheries, University of Washington, Seattle 5, Washington.

DISCUSSION

No work was done to ascertain the viability of spores. A very interesting and worthwhile study on this species could be carried out with regard to the tolerance

TABLE 2.—*Dimensions of spores, formalin-fixed, Giemsa-stained*

Character	Mean (microns)	Standard deviation	Range (microns)	Number examined
Valves				
length	7.4	0.45	6.4–8.5	110
breadth	6.6	0.42	5.9–7.4	110
Polar capsule				
length	3.7	0.30	3.2–4.3	110
width	2.5	0.30	2.1–3.2	110

of spores to refrigeration, and the incidence of subsequent infection because of the practice of some hatchery men of using spawned-out silver salmon in the diet of hatchery fish. Difficulties involved in attempting to stain the sporoplasm of spores may give some indication of the ability of the organism to resist adverse conditions.

The size at which rainbow trout and silver salmon become infected, and the manner in which they become infected, are interesting problems on which no concrete information can be offered here. Judging from the small size of the infected rainbow trout, the life cycle can probably run to completion in about 7 months. In silver salmon it would seem logical that the young in fresh water receive the infection from spawning adults. If, however, the life cycle is shorter than 15 months in silver salmon then the infection must be picked up while the adults are in the salt-water stage of their existence. Whatever spores are not shed at that time are carried into streams, giving rainbow trout the opportunity to become infected.

Two of the trout farms may receive the infection from their water supply, a small river, which supports a run of silver salmon. The ocurrence of the disease at the trout farm near Olympia is conceivably due to the placing of infected fish in the ponds, since the water supply comes from a spring and is undoubtedly free from myxosporidians when it enters the ponds. The nature of the disease precludes suggestions as to therapeutic or prophylactic measures.

SUMMARY

A new species of myxosporidian parasite *Myxosoma squamalis*, from rainbow trout, silver salmon, and chum salmon, is described. This parasite in the trophozoite stage apparently attacks the surface of the scales of these fish and in some manner forces its way inside the scales and sporulates.

It is believed that as a result of the infection the epithelial layers and the fibroblasts of the scale pocket are modified in an area closely associated with an infected scale. That the parasite is a cause of mortality in the host fish is not known.

Measurements and a description of fresh spores are given, as well as measurements of spores fixed in formalin and stained in Giemsa. A substantial shrinkage was noted as a result of the fixing and staining. The size of the trophozoite varies with the size of the scale. Fresh sporonts are described, and these may contain 1 or 2 developing spores.

ACKNOWLEDGMENTS

The author wishes to express gratitude to Dr. J. E. Lynch, of the School of Fisheries, University of Washington, for direction and criticism throughout this work and for making available material from his collection. Indebtedness is acknowledged to Dr. R. R. Rucker of the Fish and Wildlife Service, for cooperation and helpful advice. Information concerning nematodes and their identification were kindly furnished by Dr. P. V. Gustafson of the University of Washington faculty. Mr. C. Schroeter and Mr. G. M. Southward gave generously of their time and effort to produce the photomicrographs and the photographs, respectively, presented here.

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Plate I

Fig. 1. Photograph of a 12.4 cm. rainbow trout showing the typical appearance of a heavy infection within the scales.

Fig. 2. Photomicrograph of a longitudinal section of skin just anterior to dorsal fin of 15.3 cm. rainbow trout, showing the number of spores (S_1) within scale (S) and thinning of epidermis (E) with loss of mucus-secreting cells (M) above infected area. Melanophores (P), which appear as large black cells, may be seen in the dermis (D) and epidermis. 15 microns. ×129.

Fig. 3. Photomicrograph of transverse section of skin from belly of 15.3 cm. rainbow trout, showing spores (S_1) within scale (S) and what appears to be modified host tissue (T) beneath scale and above dermis (D). 5 microns. ×258.

Fig. 4. Disporoblastic sporont with nearly mature spores, Giemsa-stained. ×2475.

PLATE I

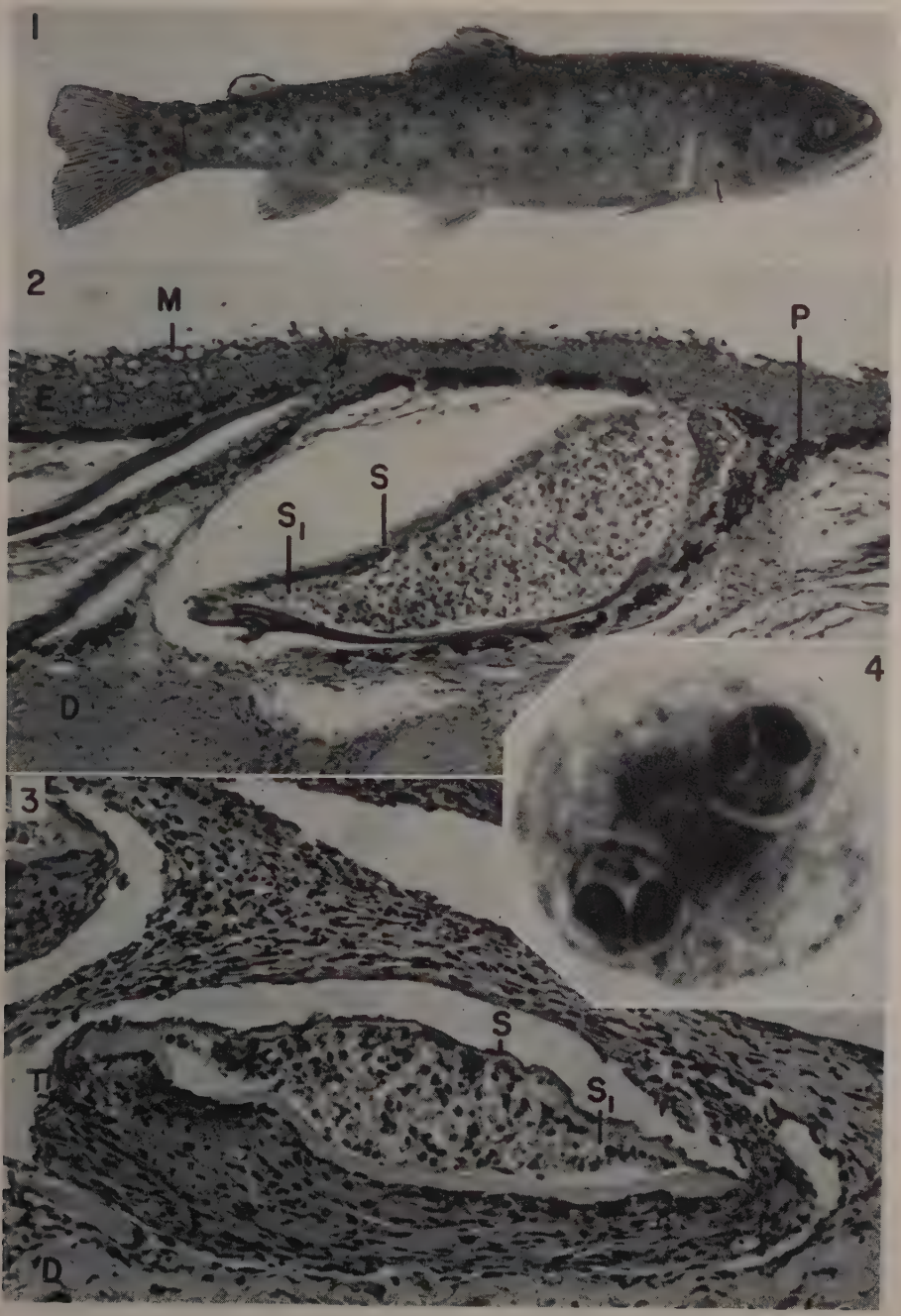

PLATE II

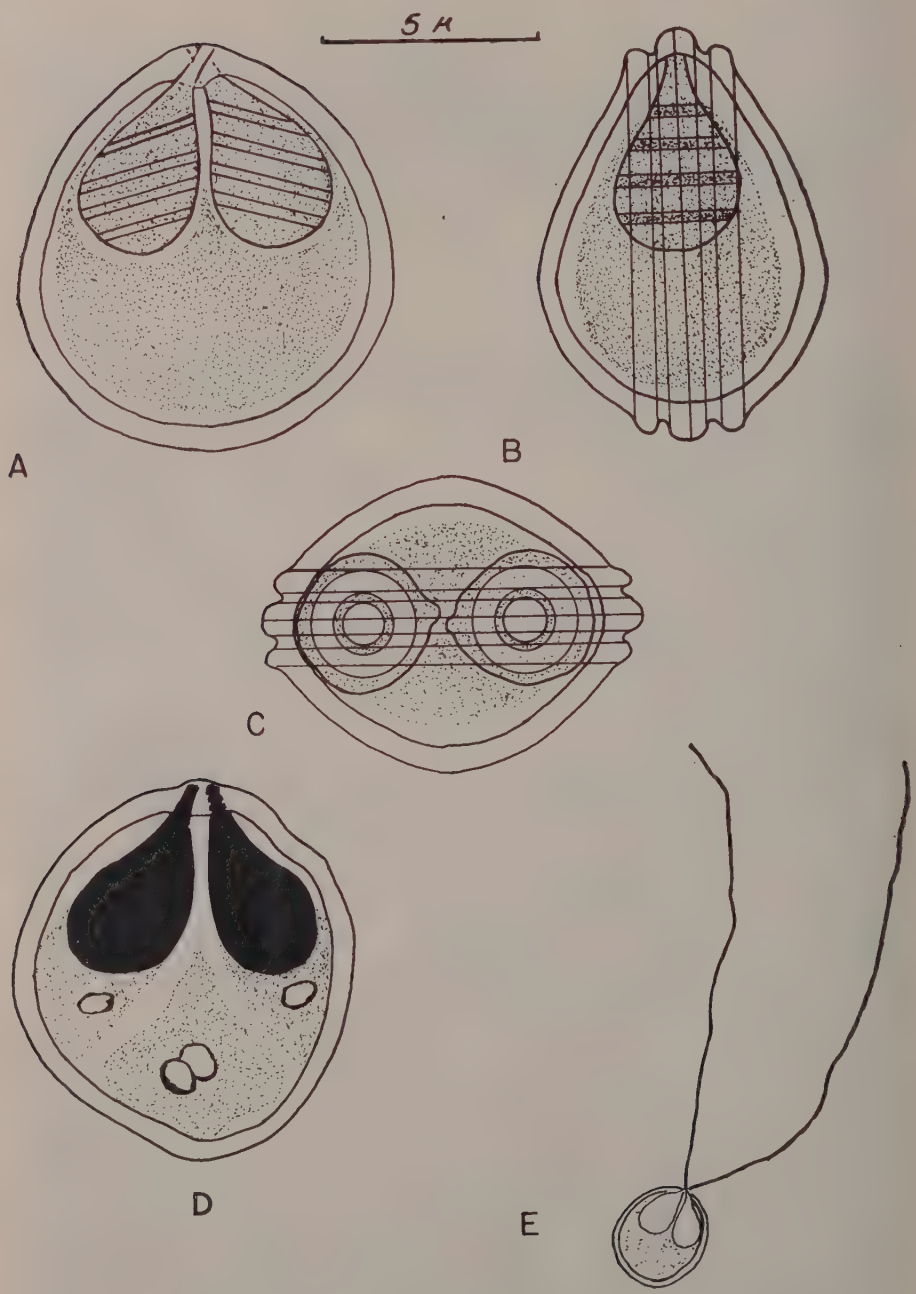

FIG. 5. Spores. (A) Front view, fresh spore, × 5644. (B) Side view, fresh spore, × 5644. (C) End view, fresh spore × 5644. (D) Front view, immature spore stained with Giemsa stain, × 4720. (E) Front view, fresh spore with polar filaments everted, × 1411.

THE PREDOMINANCE OF SINGLE-BROOD INFECTIONS IN HUMAN ASCARIASIS

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Ascariasis is acquired by the ingestion of soil containing infective eggs of *Ascaris lumbricoides*. In the United States the infection is primarily a childhood disease, Cort, *et al.*, 1930, Headlee, 1936, Beaver, 1952). It therefore appears probable that between the ages of 1 and 4 years. This is apparently due to the geophagous habit of children of this age rather than to the acquisition of an immunity as the child grows older, since adults are commonly infected in areas where the opportunity for ingestion of eggs exists. Not only can a history of dirt-eating be elicited in the case of most *Ascaris* infections but frequently the eggs of *Ascaris* can be recovered from the soil of the area in which the child is known to play (Brown, 1927, Cort, *et al.*, 1930, Headlee, 1936, Beaver, 1952). It therefore appears probable that during favorable seasons these children may ingest numbers of infective eggs almost daily. It is a matter of some interest, whether a large percentage of these eggs can develop on successive occasions and thus massive infections can be acquired, or whether massive infections result only from the ingestion of a large number of eggs at once or during a short period. For convenience worms derived from eggs ingested at about the same time will be referred to as a "brood."

Observations by Dr. Paul C. Beaver since 1947 and by the writer since 1948 on the pediatric wards of Charity Hospital, where there is about 20 percent prevalence of ascariasis, have suggested that with few exceptions infection with a single brood of *Ascaris* is the rule. The present report is intended to call attention to this fact, which, though probably recognized by many parasitologists and physicians, appears not to have been placed on record.

Ascaris lumbricoides were obtained from a total of 7 patients with massive infection either by medical treatment, or at surgery for removal of worms causing intestinal obstruction, or at autopsy. The worms were measured and their sexes determined.

Table I lists the manner in which the worms were acquired and the mean, median and range of lengths of worms in each case. The curves representing the frequency distribution of lengths, based on measurements of 50 or more worms, were all fairly symmetrical, with a single mode, and of limited spread. Worms of a single infection tended to be of one size, although there was considerable variation in the size of worms from patient to patient, depending presumably in part on the age of the infection. In case I the worms had barely become sexually differentiated. These data are indicative of a single brood of worms in the intestine.

These observations suggest that consistently there is but a single brood of intestinal *Ascaris*, even in the heavy infections. This phenomenon was previously observed in a single case by Otto (1931), who concluded that there is a rapid turnover in *Ascaris* infection resulting from the massive acquisition and spontaneous loss of worms. Although children with ascariasis spontaneously pass worms, (Otto,

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1930, Cort, Schapiro, Riley and Stoll, 1929), they do not often pass them in such numbers as to eradicate completely a bowel infection except over a period of several months. It is probable that meanwhile new broods of larvae fail to mature until the older worms are almost totally eliminated.

The inhibitory influence of successive infections with ascarids upon each other has been reported by Sadun (1948), who studied *Ascaridia galli* in the chicken. He found that the host response to a second infection may drive out the first, or may interfere with the development of the second. Beaver (1952) working with natural infections of *Ascaris lumbricoides* in the pig observed the consistent occurrence of worms of about the same size in the bowel of a single animal in all but 4 of 71 infected pigs. Although these results cannot be applied to pigs in general the writer is not aware of published evidence to the contrary based on actual measurements.

Sprent and Chen (1949) have shown that an infection with *Ascaris* larvae in experimental animals confers a type of immunity which tends to retain subsequent larval infections in the liver. The retarding influence is perhaps the tissue reaction,

TABLE 1.—*Lengths of worms in individual massive* Ascaris *infections*

Case	Manner collected	Total	Female worms Lengths (cm.)				Male worms Lengths (cm.)			
			Number	Mean	Median	Range	Number	Mean	Median	Range
I	Autopsy	570	278	20.7	18.5	9.0 – 30.0	165	13.7	13.5	7.0 – 21.5
II	Treatment	63	35	20.5	20.5	15.0 – 25.0	28	14.5	14.5	10.0 – 19.0
III	Surgery	93	44	22.5	22.0	19.0 – 27.0	49	15.7	16.0	9.0 – 19.0
IV	Treatment	52	29	19.7	18.5	13.0 – 24.5	23	13.7	14.0	11.5 – 19.0
V	Autopsy	246	151	19.0	19.0	13.0 – 25.5	95	14.0	14.0	10.5 – 18.0
VI	Treatment	56	33	21.4	21.0	15.0 – 33.5	23	11.8	14.0	12.0 – 22.0
VII	Treatment	129	95	11.3	10.5	7.0 – 14.0	34	8.9	9.0	6.0 – 12.0

which increases in intensity with successive infections (Sprent and Chen, *l.c.*, Kerr, 1938).

The fate of *Ascaris* larvae entering the body during an intestinal infection has not been studied. They may complete their migration and then be eliminated without maturing, may become ensnared as in Sprent's reinfection studies in abnormal hosts or may perhaps persist for a time but with reduced activity due to host tissue reaction. These *Ascaris* larvae may thus be capable of producing a disease similar to the visceral larva migrans produced by *Toxocara* (Beaver, Snyder, Carrera, Dent and Lafferty, 1952), a potentiality which is, however, limited by the relatively greater susceptibility of *Ascaris* to encapsulation and destruction by the host (Hoeppli, Feng and Li, 1949).

SUMMARY

In 7 cases of heavy *Ascaris lumbricoides* infection in human beings the worms were all collected and measured. The sizes of worms in each infection indicated that the worms were of about the same age. This suggests that a pre-existent intestinal infection with *Ascaris* may prevent the development of worms entering the body subsequently.

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A NEW CHIGGER MITE (*GENUS EUSCHÖNGASTIA*) FROM THE CENTRAL UNITED STATES[1, 2]

LOUIS J. LIPOVSKY AND RICHARD B. LOOMIS

Studies of the chigger fauna of Kansas have revealed a species of chigger mite of the genus *Euschöngastia* described below, which is distinct from, but closely related to, the bat chigger, *Euschöngastia pipistrelli* Brennan, 1947. The terminology used throughout this paper is that of Wharton, *et al.,* 1951. All measurements are in microns.

We wish to thank Mr. Robert B. Finley, Jr., Mr. J. Knox Jones, Jr. and Mr. Ervin H. Kardos for aid in obtaining larvae and their hosts. Thanks are also extended to Mr. D. A. Crossley, Jr. and Dr. Charles D. Michener of the University of Kansas for reading the manuscript.

Euschöngastia jonesi[3] new species

[3] Named for Mr. J. Knox Jones, Jr., formerly at the University of Kansas, who caught the host from which the type series was obtained, and who has aided in the collection of many other chiggers and their hosts.

Diagnosis.—Closely related to *Euschöngastia pipistrelli* in having 1 tibiala III; 2 genualae I; subterminala and parasubterminala I; scutum twice as wide as long, with long posterolateral setae; palpal claw with 5 prongs; and lateral palpal tibial seta nude. It differs from the above mentioned species in having the galeal seta branched; sensilla clavate, gradually tapering distal part with a few large setules; and distinct eyes 2/2.

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[1] The studies upon which this paper is based were conducted under a contract, N6 ori 220 Task Order 2, between the University of Kansas and the Office of Naval Research.

[2] Contribution No. 864 of the Department of Entomology, University of Kansas.

Description of larva.—Body: Holotype (unengorged), 246 by 238 (engorged 802 by 483); white in life. Eyes, 2/2, pink in life, anterior slightly larger; length of both on 1 side 18, ocular plate faint.

Dorsal setal formula 2-13-13-13-14-10, total 64 (some paratypes with 14 setae in first and second rows); humeral seta measures 72, anterior dorsal seta 68, posterior dorsal seta 56. Ventral setal formula approximately 2-2-12-15-10-10-11-10-2, total 74; anterior sternal seta measures 54, posterior sternal seta 40, anterior ventral seta 25, posterior ventral seta 42. Total body setae approximately 138.

Scutum: Width more than twice the length, punctae small and scattered, posterior margin slightly rounded with a slight central concavity, a crescent-shaped ridge over each sensillary base, not meeting in center; bases of anterolateral and posterolateral setae situated on slight scutal projections, PL's long, AM and AL's approximately the same length, sensillary bases posterior to bases of the PL's. Sensilla clavate, tapering distally to a point, setules scattered, larger and decreasing in number distally. Scutal measurement of holotype: AW—67, PW—92, SB—34, ASB—31, PSB—12, AP—24, AM—49, AL—48, PL—83, S—45, (width 11). Average and extremes of 11 larvae both unengorged and engorged, 5 from Cowley, 2 from Douglas, and 4 from Barber County, Kansas; AW—67 (63–73), PW—92 (83–106), SB—33 (28–37), ASB—30 (26–33), PSB—12 (11–14), AP—24 (21–28), AM—45 (43–49), AL—47.5 (43–53), PL—90 (83–103), S—42 (41–46).

Gnathosoma: Chelicera blade long and stout, one tricuspid dorsal cap; base punctate. Galeal seta with 3 branches. Capitular sternum punctate, with a single pair of branched setae. Palpal femur and genu each with 1 branched seta, tibia with branched dorsal and ventral setae and nude or single-branched lateral seta; tarsus with a basal spur (10), and 7 branched setae. Palpal claw with 5 prongs, 2 short prongs and 3 long prongs of approximately equal length (see fig. 1).

Legs: Leg I coxa, trochanter and basifemur each with 1 branched seta; telofemur with 5 branched setae; genu with 4 branched setae, 2 genualae and a microgenuala; tibia with 7 branched setae, 2 tibialae and a microtibiala; tarsus with approximately 20 branched setae, a spur (18), microspur, subterminala, parasubterminala, and pretarsala. Leg II coxa and trochanter each with 1 branched seta; basifemur with 2 branched setae; telofemur with 4 branched setae; genu with 3 branched setae and a genuala; tibia with 6 branched setae and 2 tibialae; tarsus with 17 branched setae, spur (18), microspur, and a pretarsala. Leg III coxa and trochanter each with 1 branched seta; basifemur with 2 branched setae; telofemur with 3 branched setae; genu with 3 branched setae and a genuala; tibia with 6 branched setae and a tibiala; tarsus with approximately 15 branched setae. Setules reduced in number on setae of distal segments of all legs. All leg segments with scattered punctae.

Remarks.—Euschöngastia jonesi n. sp. has been taken on bats but never in the same locality as *E. pipistrelli.* The type host was captured at night in a small limestone cave. All of the hosts were taken in rocky habitats, limestone occurring in the east, sandstone and gypsum in the west.

The larvae were found in and on the ears of the hosts. The type larvae were scattered over the face and ears of the white-footed mouse.

This species has been reared to the nymphal stage by one of us (Lipovsky) from larvae taken in Barber County, and by Mr. D. A. Crossley, Jr., from larvae taken with the type series. The latter nymphs were individually correlated with cast larval skins.

Types.—Larvae: Holotype, slide no. 6930 and 25 paratypes, nos. 6931–55, Snow Entomological Museum, University of Kansas, from 2 miles east of Rock (14 miles north of Winfield), Cowley County, Kansas, taken from a white-footed mouse, *Peromyscus leucopus* (Rafinesque), field number RL530307-5, obtained on March 7, 1953 by J. Knox Jones, Jr. and Olin L. Webb.

Paratypes will be sent to the United States National Museum; Rocky Mountain Laboratory; British Museum (Natural History); Dr. George W. Wharton; The South Australian Museum, Adelaide; Dr. Charles D. Radford; the Museum National d'Histoire Naturelle, Paris, France; Army Medical Service Graduate School, Washington, D. C. and Dr. J. R. Audy, Institute for Medical Research, Kuala Lumpur, Malaya.

Additional specimens examined.—Total, 69 larvae, as follows. KANSAS. *Douglas County*: 5 mi. N, 1 mi. E Lawrence, Univ. Kansas Nat. Hist. Reservation, *Cryptotis parva,* Oct. 16, 1951, KU 6964–75 and chigger sampler, Nov. 9, 1951, KU 6976; 5 mi. S, 5 mi. W Lawrence, *Peromyscus leucopus,* April 28, 1952, KU 6977–78 (total 4 specimens); 2 mi. S, 2 mi. W Pleasant Grove, *Sylvilagus floridanus,* Dec. 4, 1948, KU 6979, Dec. 29, 1948, KU 6980–81, Jan. 29, 1949, KU 6982. *Cowley County*: 2 mi. E Rock, *Peromyscus leucopus,* March 7, 1953, KU 6955–63 (total 18). *Barber County*: 5 mi. S Sun City, *Peromyscus maniculatus,* April 12, 1949, KU 6984–89; 4 mi. S Aetna, *Neotoma micropus,* April 11, 1949, KU 6983, *Myotis velifer,* April 13, 1950, KU 6990–91, *Peromyscus maniculatus,* Oct. 7, 1951. KU 6992–98. OKLAHOMA. *Woods County*: 6 mi. S, 2 mi. W Aetna, Kansas, Merrihew Cave, *Myotis velifer,* Oct. 6, 1951, KU

6992–7002. MISSOURI. *Lawrence County*: 0.5 mi. S Verona, *Peromyscus leucopus*, Sept. 6, 1947, KU 7003–06.

SUMMARY

A new species of chigger mites, *Euschöngastia jonesi*, is described from various localities in Kansas, Oklahoma and Missouri.

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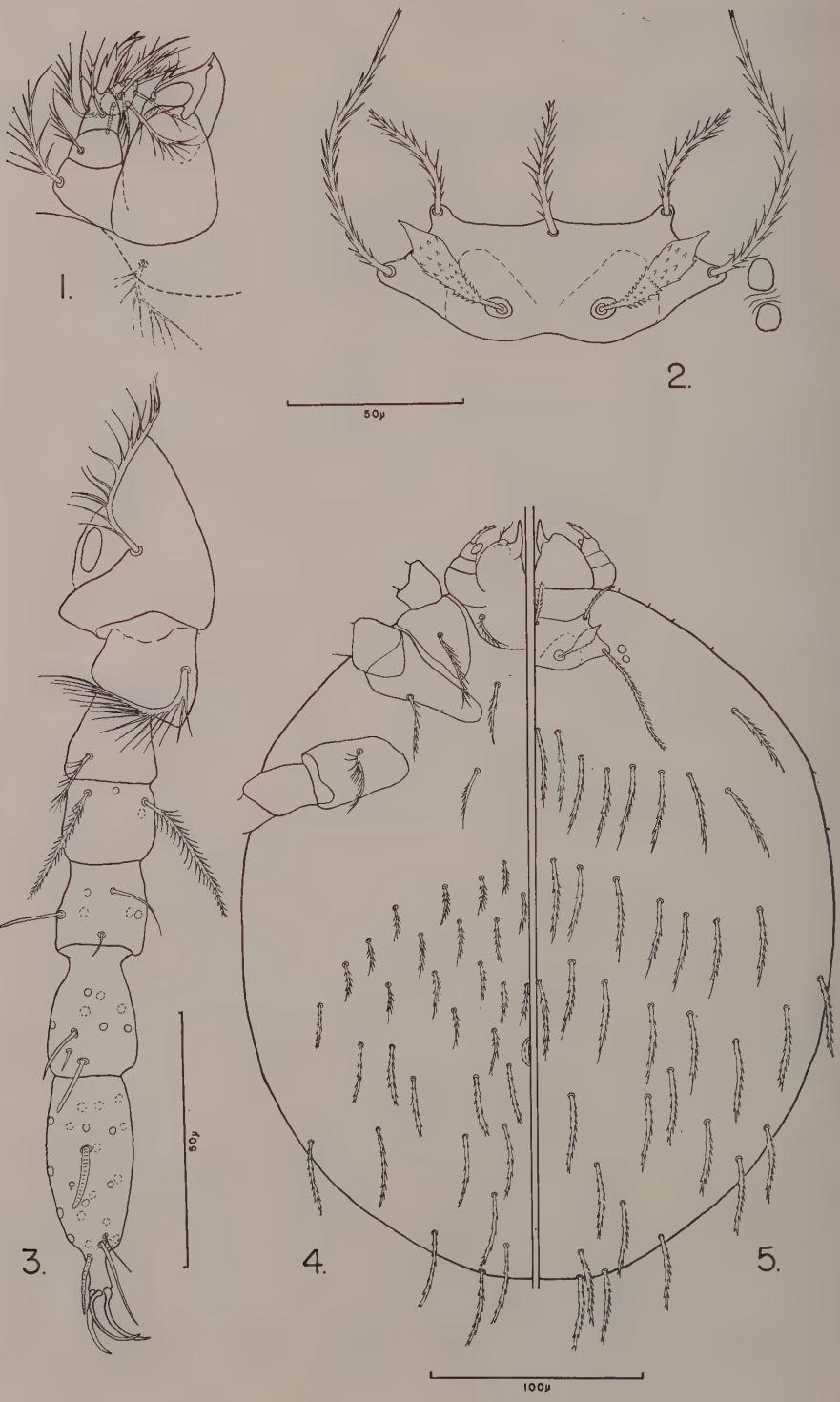
EXPLANATION OF PLATE

Euschongastia jonesi new species

FIG. 1. Dorsal aspect of gnathosoma.
FIG. 2. Scutum and eyes.
FIG. 3. Leg I, showing all nude setae.
FIG. 4. Ventral aspect of body.
FIG. 5. Dorsal aspect of body.

PLATE I



EXOGENOUS PROLIFERATION IN A LARVAL TAENIID (CESTODA: CYCLOPHYLLIDEA) OBTAINED FROM THE BODY CAVITY OF PERUVIAN RODENTS

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The occurrence of asexual larval multiplication in cestodes has been reported by various authors, and in recent years notably by Dollfus (1938, 1951). While endogenous proliferation occurs normally among members of the family Taeniidae, exogenous multiplication is less frequent and may in some instances represent an abnormal development (Kuntz, 1943).

The evidence presented below indicates that exogenous larval proliferation may be a part of the normal development of some taeniids. The description which follows is based on specimens obtained from the body cavity of three *Ctenomys peruanus* collected December 17, 1951, and February 5, 1952, at Pampa Queullecota, 13,000 ft., 60 km. S. Ilave, Department of Puno, Peru; from one *Phyllotis osilae* collected February 9, 1952, 15 km. W. Puno, 13,000 ft., Department of Puno, Peru; and from one *Chinchillula sahamae* collected January, 1952, 5 km. E. Lago Suche, 14,600 ft., Department of Moquegua, Peru.

I am very grateful to Dr. and Mrs. Oliver P. Pearson, Museum of Vertebrate Zoology, University of California, Berkeley, for collecting these interesting specimens for me. Thanks are due to Mr. Zane Price, Department of Infectious Diseases, School of Medicine, University of California, Los Angeles, for the photograph in Plate I.

DESCRIPTION

The larvae from the three rodent hosts are fairly uniform in external appearance. Individual cysticerci vary from 9 to 15 mm. in length, and from 3 to 4 mm. in width; contracted specimens are usually less than 9 mm. long. The scolices are invaginated. At the posterior end, each cysticercus bears a slender stalk of variable length. In some groups of larvae, the stalk is confluent with a bladder from which the individual cysticerci proliferate; in other groups the point of attachment consists of a mass of dense tissue (Fig. a). The number of cysticerci originating from each bladder, varied from nine to 20 in presumably full grown specimens. Figure a illustrates such a group in which some of the larvae are contracted and others relaxed; all cysticerci are apparently derived from a single bladder.

A few specimens from *Chinchillula sahamae* suggest that the first step in the development of the group is the growth of one cysticercus which bears a relatively narrow and transparent bladder at its posterior end; from this bladder additional larvae proliferate. In such relatively "young" larval groups one may see individual cysticerci varying in length from 1 to 10 mm. or more; a bladder-like structure is always present. In other groups which consist of individuals uniform in size, the bladder is usually much smaller or absent entirely. In its place, there is a variable amount of stringy and hardened tissue (Fig. a).

Hook size and number vary considerably, depending on the stage of development of the specimen examined. In apparently fully grown cysticerci, the number of

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hooks was 40–44; of these, 20 or more measured 120–140 microns (Fig. c), and alternated in position with a similar number of larger hooks measuring 190–220 microns in length (Fig. b).

I have previously reported different data for hook size and number of these cysticerci (Voge, 1953). It appears from a more thorough investigation of the material, that the data in my earlier report were obtained from larvae which were not fully grown.

DISCUSSION

In external appearance, the cysticerci from the Peruvian hosts resemble the description given by Dollfus (1951) of taeniid larvae recovered from the pleural cavity of *Gerbillus pyramidum* in Africa. The Peruvian material differs from that described by Dollfus in the number, size and shape of the hooks, which are much smaller and less numerous in my specimens. Dollfus (1951) includes a table of data recorded by other authors who described exogenous proliferation in taeniid larvae thought to be *Taenia taeniaeformis* (Bloch, 1780). In all of these, the hooks are considerably larger than they are in my specimens. Schwartz (1924) described and illustrated an exogenously proliferating cysticercus, *Taenia twitchelli*, from the lungs of a porcupine but his species bears little resemblance to the Peruvian specimens. It should be stressed that hook size varies with the stage of development of the larva; comparisons are of little value unless based on hooks which have reached their maximal development within the cysticerci. External structure, particularly the shape of individual cysticerci and the mode of branching, might in some instances be a more reliable criterion for differentiation.

Specific identification of the Peruvian cysticerci described herein, cannot be made until more is known about the adult taeniids in the High Andes. Further indication that exogenous proliferation is a regular occurrence in this species is evident in the observations of Dr. and Mrs. Pearson, who recorded these larvae from at least six *Ctenomys peruanus* and noted them in several other individuals of the same host species.

SUMMARY

Exogenous proliferation is described in taeniid larvae obtained from the body cavity of three species of Peruvian rodents. This type of asexual, larval multiplication was observed in all specimens studied and is therefore thought to be a normal part of the development of this cestode. In view of our scanty knowledge of adult taeniid cestodes in Peru, specific identification or description is at present not desirable.

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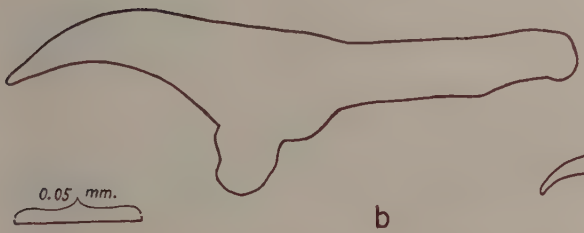
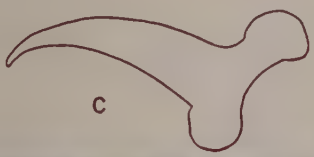
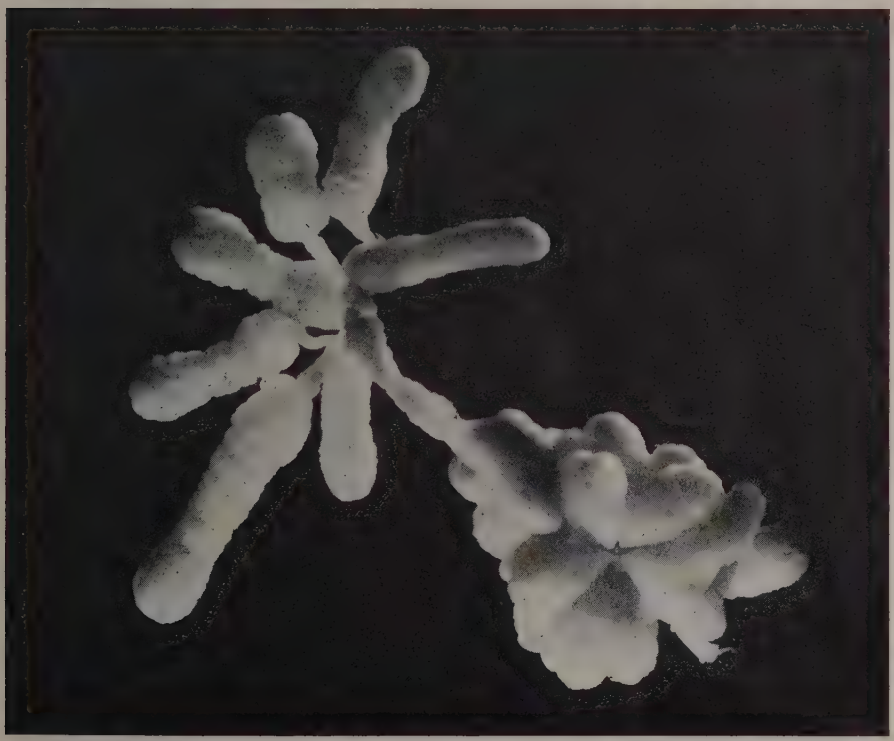
PLATE I







EXPLANATION OF PLATE

Drawing made with the aid of a camera lucida.

PLATE I

FIG. a. Photograph of a group of cysticerci from the body cavity of *Ctenomys peruanus;* magnification a little over 3× original size.

FIG. b. Large hook.

FIG. c. Small hook.

SOME FACTORS AFFECTING THE INTENSITY OF *EUHAPLORCHIS CALIFORNIENSIS* (TREMATODA: HETEROPHYIDAE) INFECTIONS IN CHICKS[1]

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The purpose of this investigation was to determine factors affecting the intensity of infection of *Euhaplorchis californiensis* in chicks by variation in diet and period of starvation of the host.

Infections of *Euhaplorchis californiensis* have been experimentally established in the small intestine of chicks (Martin, 1950); in cats (Bamberger and Martin, 1951); in monkeys (Bamberger and Martin, unpublished); and in lizards and bats (Meniskes and Martin, unpublished). The life cycle of the trematode was described by Martin (1950).

Few previous reports have compared the number of metacercariae fed and the number of adult flukes developed to maturity in the definitive host. In a study of the resistance of metacercariae (*Haplorchis taihokui*) to various methods of preparing raw fish for human consumption in the Philippine Islands, Garcia (1936) recovered 22 to 23 flukes for each 30 metacercariae about 2 weeks after they were fed to control mice. Bamberger and Martin (1951) recovered 2,511 (about 12%) *Euhaplorchis californiensis* adults from a control cat fed fish brains containing approximately 20,000 metacercariae and sacrificed 10 days after the initial feeding.

Some studies have been made to determine the effects of the host's diet on resistance to cestode and nematode parasites, but no reports on trematodes have been found. Zimmerman, Vincent and Ackert (1926), Ackert, Fisher and Zimmerman (1927), Ackert and Spindler (1929) and Ackert and Nolf (1931) showed that a deficiency in certain vitamins lowered the resistance of chickens to the nematode, *Ascaridia lineata.* Clapham (1933, 1934) showed that vitamin A increased the resistance of chicks to *Heterakis gallinae* while a diet deficient in mineral content lowered resistance to that parasite.

Ackert and Beach (1933), Ackert, Branson and Ameel (1944), Ackert (1947), Riedel (1946) and Riedel and Ackert (1950) found that inclusion of skim milk in the diet increased the resistance of the chicks to the nematode, *Ascaridia lineata.* Becker (1933) found that a milk diet caused the cessation of egg production of the cestode, *Hymenolepis nana,* in the rat but did not kill the worms.

Starvation of the host also affects helminths. Levine (1938) found that there was a marked decrease in proglottid production of *Davainea proglottina,* when the chick hosts were fed rations with little or no food value. No decrease in egg production by *Hymenolepis diminuta* was noted by Hager (1941) who fed the rat host a partial starvation diet by reducing the food intake to one-third of the normal

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[1] Part of a dissertation submitted to the University of Southern California in partial fulfillment of requirements for the degree of Doctor of Philosophy. The writer is indebted to Dr. W. E. Martin, University of Southern California, for his guidance and advice throughout the investigation.

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amount. Reid (1940) reported that a starvation period of 20 to 48 hours resulted in the breaking off of long chains of proglottids of the fowl cestode, *Raillietina cesticillus*. He concluded that the effects of starvation on proglottid production was due to lack of carbohydrates. Reid and Ackert (1941) also found loss of strobilae of the same cestode in partial starvation of the host.

MATERIALS AND METHODS

Day-old New Hampshire chicks were kept at 32° to 34° C. and given no food or metacercariae until they were at least 24 hours old. The chicks were separated into 3 groups with 36 in Group I, 24 in Group II and 6 in Group III. Group I chicks were subdivided into 6 classes: A, B, C, D, E, and F, and were given an all-purpose feed. Group II chicks were given ground oatmeal and were subdivided into 4 classes: A, B, C, and D. Each class consisted of 6 chicks. The chicks in Class A of both groups had access to food at all times; those in Class B of both groups were fasted 5 hours before the feeding of metacercariae. Class C of both groups fasted for 5 hours after the feeding of metacercariae. In Class D of both groups, the chicks were given no food for 5 hours before and 5 hours after the feeding of metacercariae. Class E chicks fasted 10 hours after the feeding of metacercariae, and Class F chicks fasted 15 hours after the ingestion of metacercariae.

In Group III, 6 chicks were given powered milk. They were given no food for 5 hours following the feeding of metacercariae.

All chickens were given 1 feeding of 500 metacercariae of *Euhaplorchis californiensis* removed from the brains of naturally infected fish, *Fundulus parvipinnis parvipinnis*, collected in a brackish canal at Playa del Rey, California. These metacercariae were isolated in physiological salt solution for cold-blooded animals, were counted, and then pipetted down the throat of each chick. The chicks were fed the feed indicated for each group 24 hours after hatching, and were given metacercariae between the second and tenth day of life. Thus, all hosts were fed prior to infection. Since the flukes become adults in 4 to 6 days, all chicks were sacrificed 6 days after the feeding of metacercariae. The hosts were fasted overnight before being sacrificed in order to decrease the amount of intestinal contents which might interfere with the search for the parasites. The intestines were cut into two-inch sections and the worms recovered and counted.

RESULTS AND DISCUSSION

The first 4 classes of Group II consisted of 24 chicks which received chick feed. Each chick was given 500 metacercariae, or a total of 12,000 metacercariae. A total of only 34 flukes were recovered, or an average of 1.4 flukes per chick. However, from the chicks of the 4 classes of Group I which received oatmeal 1,120 flukes were recovered, or an average of 46.6 worms per chick. Thus the number of adult parasites recovered from chicks fed oatmeal exceeded that recovered from chicks fed all purpose chick feed (Table 1).

There were variations also in the numbers of parasites recovered from chicks kept on the same diet, but with fasting periods of different duration. In hosts receiving chick feed, the number of flukes recovered varied from 0 to 9. The number recovered from those receiving oatmeal varied from 0 to 301 flukes. These variations depended upon whether the chicks had fasted and the number of hours of fasting.

The smallest number of flukes recovered was from the chicks which had access to food at all times. The greatest number of worms was recovered from chicks which had no access to food for 5 hours after the feeding of metacercariae. The average number of flukes remained approximately the same in oatmeal-fed chicks that were starved for 5 or 10 hours after the feeding of metacercariae, and decreased in chicks which were fasted for 15 hours. The number of worms recovered from chicks fasted 5 hours before and 5 hours after the feeding of metacercariae was smaller than the number recovered from chicks fasted for only 5 hours after ingesting the cysts. The number of flukes recovered from chicks starved for 5 hours before the feeding of metacercariae was smaller than the number recovered from those starved for 5 hours after ingesting the cysts.

TABLE 1.—*Difference in intensity of fluke infection in chicks due to different food and varying periods of fasting. Each chick was given 500 metacercariae*

	FEED														
	Group I All Purpose Chick Feed					Group II Oatmeal					Group III Powdered Milk				
			Flukes recovered					Flukes recovered					Flukes recovered		
Fasting Condition	No. of chicks	Age in days	Total no.	%	Av. no. per chick	No. of chicks	Age in days	Total no.	%	Av. no. per chick	No. of chicks	Age in days	Total no.	%	Av. no. per chick
Class A No fasting	6	2–10	3	0.1	0.5	6	2–10	20	0.6	3.3					
Class B Fasting 5 hrs. before feeding of metacercariae	6	2– 7	3	0.1	0.5	6	2– 6	143	4.7	23.8					
Class C Fasting 5 hrs. after feeding of metacercariae	6	2– 5	17	0.6	2.8	6	2– 6	650	21.7	108.3	6	3–9	64	2.1	10.7
Class D Fasting 5 hrs. before and 5 hrs. after feeding of metacercariae	6	4– 7	11	0.4	1.8	6	3–10	307	10.2	51.2					
Class E Fasting 10 hrs. after feeding of metacercariae						6	2– 3	616	20.5	102.7					
Class F Fasting 15 hrs. after feeding of metacercariae						6	2– 3	372	12.4	62.0					

Of the 6 chicks fed only powdered milk and fasted for 5 hours after the feeding of 500 metacercariae each, 4 to 29 flukes were recovered from 4 chicks and none from the other 2. This count is much lower than for chicks fed oatmeal and is a little higher than for those fed chick feed.

The content of food in the chick gut apparently interfered with excystment or with attachment of flukes to the host. Chicks which had free access to food (oatmeal or chick feed) on the day that the metacercariae were ingested yielded few flukes. Starving the host for 5 to 10 hours after receiving metacercariae probably allowed the worms to attach themselves at their usual position in the lower part of the intestine and thus increased the number of parasites recovered from the chicks.

The present experiment indicates that a deficient diet and fasting of the host for a short period after the feeding of metacercariae are factors enabling *E. californiensis* to develop in greater numbers in an unnatural host such as the chick.

All adult flukes were recovered from the last 10 inches of the intestine, and most of them from the last 4 inches of this segment; but some were occasionally recovered from the ceca and, less frequently, the rectum. Of the total of 3,259 flukes recovered from 113 chicks fed 500 metacercariae each: 5 per cent were found in the rectum, 10.5 per cent in the ceca, 28 per cent in the segment 0 to 2 inches anterior to the rectum, 35 per cent in the segment 2 to 4 inches anterior to the rectum, 18.4 per cent in the segment 4 to 6 inches anterior to the rectum, 2.9 per cent in the 6 to 8 inch segment, 0.2 per cent in the 8 to 10 inch segment anterior to the rectum, and none anterior to this last segment. See figure 1.

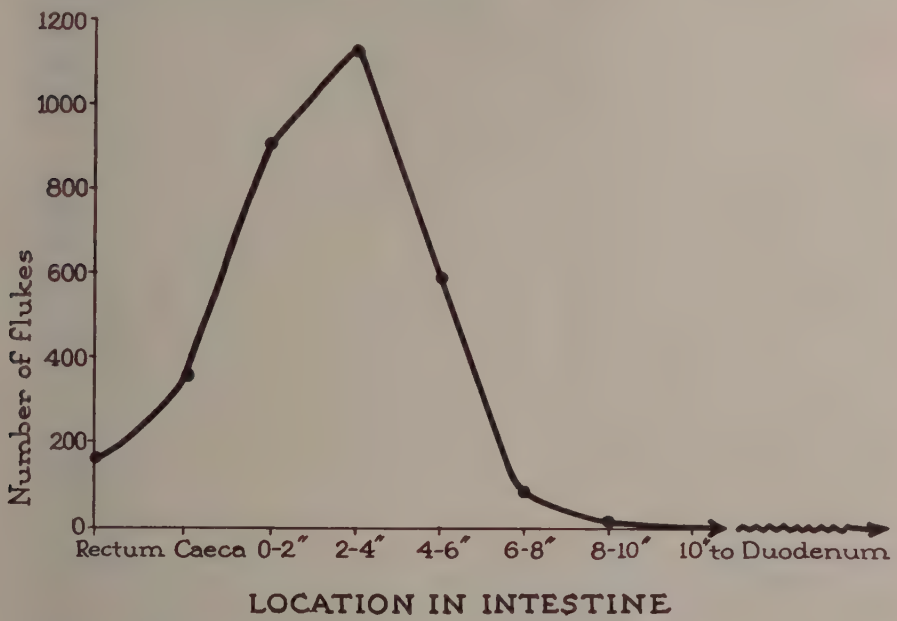

FIG. 1. Relationship of the number of flukes to their location in the rectum, caeca, and 2-inch segments of intestine forward.

Five hours after 1 chick was fed 500 metacercariae, 82 per cent of the total number of flukes present occurred in the last 4 inches of the intestine anterior to the rectum. In 2 other chicks fed 20 infected fish brains each and sacrificed 5 hours later, the majority of flukes recovered also occurred in the 4 inches of intestine anterior to the rectum. Thus, the majority of the worms became located in the posterior portion of the intestine very early after an infective feeding of the host.

SUMMARY

1. A large number of adult *Euhaplorchis californiensis* were recovered from chicks which were fed oatmeal than from those fed an all-purpose chick feed.

2. Allowing the birds to have access to food at all times rendered conditions less favorable for the development of *Euhaplorchis californiensis*.

3. Chicks fed an oatmeal diet and fasted for 5 to 10 hours after the feeding of metacercariae showed the heaviest infections.

4. Fewer adult flukes were found in milk-fed chicks than those fed oatmeal.

5. Most of the worms were found in the last 4 inches of intestine.

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THE TOXICITY OF SOME ALKYL SODIUM SULFATE DETERGENTS FOR HORSE STRONGYLE (NEMATODA) EGGS AND LARVAE

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Levine and Ivens (1953) reported that two detergents, Triton NE and Tergitol 7, were toxic for the eggs and larvae of horse strongyles. The former, a nonionic detergent, is a polyethylene glycol ether, while the latter is an anionic detergent whose active ingredient is an alkyl sodium sulfate. The high toxicity of the latter observed in this preliminary study made it desirable to investigate it and other related detergents more intensively. The present paper reports the effects of three alkyl sodium sulfate detergents on the eggs and larvae of horse strongyles. An abstract of a portion of this work has been published (Levine and Ivens, 1952).

MATERIALS AND METHODS

The three anionic detergents employed in this study were (A) the sodium sulfate derivative of 3,9-diethyltridecanol-6; this was used in a 25% aqueous solution which also contained 15% of the monobutyl ether of diethylene glycol as a coupling agent and 5% of a mixture of sodium sulfate and sodium chloride; (B) the sodium sulfate derivative of 7-ethyl-2-methylundecanol-4; this was used in a 25% aqueous solution which also contained 10% of the monoethyl ether of diethylene glycol as a coupling agent and 5% of a mixture of sodium sulfate and sodium chloride; and (C) the sodium sulfate derivative of 2-ethylhexanol; this was used in a 40% aqueous solution which also contained 5% of a mixture of sodium sulfate and sodium chloride. The trade names of the three detergents above (manufactured by the Carbide and Carbon Chemicals Co., New York) are respectively Tergitol 7, 4 and 08. They will be referred to in the text as A, B and C.

The effects of these detergents on horse strongyle eggs and larvae were studied in water and in horse feces. The strongyle eggs and larvae used in this study were obtained from the feces of horses belonging to the University of Illinois. Over 95% were those of small strongyles whose third stage larvae have eight intestinal cells.

The technics used in collecting, washing and treating the eggs and larvae in water have been described by Levine and Ivens (1953). Detergent A was tested against eggs, and against first, second and third stage larvae in concentrations of 0.1, 0.05, 0.025, 0.01, 0.005, and 0.0025%. Detergent B was tested against eggs at concentrations of 0.8, 0.6, 0.5, 0.1, 0.05, 0.025 and 0.01%; against first stage larvae in concentrations of 0.3, 0.2, 0.1, 0.05 and 0.025%; against second stage larvae at concentrations of 0.5, 0.3, 0.2, 0.1 and 0.05%; and against third stage larvae at concentrations of 0.8, 0.6, 0.5 and 0.1%. Detergent C was tested against eggs at concentrations of 5.0, 1.5, 1.0, 0.8, 0.5, 0.1 and 0.05%; against first stage larvae at concentrations of 1.0, 0.8 and 0.5%; against second stage larvae at concentrations of 1.5, 1.0, 0.8 and 0.5%; and against third stage larvae at concentrations of 5.0, 4.0, 3.0 and 1.0%.

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Three treated groups of eggs or larvae were set up at each concentration; two control groups were set up at the same time. Counts were made after 1, 2, 3 and 4 days of the numbers of eggs which had embryonated, the numbers of eggs which had hatched, and the numbers of larvae which were alive. The corresponding percentages were calculated and then corrected to take into account the percentages of the control eggs which had failed to embryonate or hatch or of control larvae which had died. These percentages were relatively small. After four days, 93–97% of the control eggs had embryonated, and 92–96% of them had hatched. After one day, 98–100% of the control first stage larvae and 99–100% of the control second and third stage larvae were still alive. After four days, 60–93% of the control first stage larvae, 87–91% of the control second stage larvae, and 96–100% of the control third stage larvae were still alive.

The average number of eggs used in each treated and control group was approximately 24. The average number of first stage larvae used in each treated group was approximately 22, and that in each control group approximately 21. The average number of second stage larvae used in each treated group was approximately 22, and that in each control group approximately 23. The average number of third stage larvae used in each treated group was approximately 21, and that in each control group approximately 22.

The *t* test was used to determine whether the observed differences between the treated and control groups were significant.

The technic used in testing the compounds against the developmental stages in feces has been described in detail by Levine (1950).

RESULTS

Washed eggs and larvae in water. For reasons of space, the complete results are not tabulated. The results can be summarized in two ways: either as the lowest concentrations at which the detergents had any effect at all, or as the lowest concentrations at which they killed all, or almost all, the larvae or eggs.

In terms of the former, and using the 1% level of significance as a criterion, 17% of the eggs were prevented from embryonating by 0.01% of Detergent A, 87% by 0.5% of Detergent B, and 100% by 1.5% of Detergent C.

Hatching of 58% of the eggs was prevented by 0.01% of the Detergent A, hatching of all eggs by 0.5% of Detergent B, and hatching of 68% of the eggs by 1.0% of Detergent C.

The first stage larvae were not affected by 0.005% of Detergent A in 2 days, but this concentration killed 91% of them in 4 days. They were not affected by 0.05% of Detergent B in one day, but 65% of them were killed by it in 4 days. They were not affected by 0.5% of Detergent C in one day, but 85% of them were killed in 4 days.

The second stage larvae were not affected by 0.005% of Detergent A in 2 days, but 36% of them were killed in 4 days. Forty-four percent of them were killed in 1 day and 99% in 4 days by 0.1% of Detergent B. They were not affected in 1 day by 0.5% of Detergent C, but 76% of them were killed in 4 days.

The third stage larvae were not affected by 0.025% of Detergent A in 1 day, but all of them were killed in 4 days. Eighty-eight percent of them were killed in 1 day by 0.05% of this detergent, and all of them were killed in 2 days. Thirty-six

TABLE 1.—*Lowest effective concentrations (%) of alkyl sodium sulfate detergents on horse strongyle eggs and larvae**

	Detergent A		Detergent B		Detergent C	
	1 Day	4 Days	1 Day	4 Days	1 Day	4 Days
Prevention of egg embryonation	0.05	0.05	0.6	0.6	1.5	1.5
Prevention of egg hatching	0.025	0.025	0.5	0.5	1.0****	1.5
Killing of 1st stage larvae	0.01	0.005**	0.2	0.1	0.8**	0.8
Killing of 2nd stage larvae	0.025	0.01***	0.2**	0.1***	1.5	0.8**
Killing of 3rd stage larvae	0.1**	0.025	0.6**	0.5**	4.0**	3.0

* Except where otherwise indicated, egg embryonation and hatching were completely prevented and all larvae were killed. Significant effects in which more than 10% of the eggs embryonated or hatched or in which more than 10% of the larvae were alive are not shown in this table.
** Less than 10% alive.
*** 1% alive.
**** 10% hatched.

percent of third stage larvae were killed in 1 day by 0.5% of Detergent B, and 97% of them in 3 days. Seventy-one percent of them were killed by 3.0% of Detergent C in 1 day, and all of them in 2 days.

In Table 1 are given the lowest effective concentrations of these detergents when 90% effectiveness is used as a criterion. In general, the first stage larvae were the least resistant, followed in order of increasing resistance by the second stage larvae, eggs and third stage larvae. Somewhat lower concentrations of the detergents prevented eggs from hatching than from embyonating.

Detergent A was the most toxic of the 3 detergents, and Detergent C the least toxic. For example, in killing first stage larvae, Detergent A was almost 20 times as active as Detergent B and almost 160 times as active as Detergent C. The relative activities of the three detergents against the eggs and second and third stage larvae were quite similar.

Eggs and larvae in feces. These detergents were much less effective in feces than in water. Detergent A and Detergent C killed or prevented the development of all larvae at a concentration of 2.5% but not at a concentration of 1.0%. Detergent B killed or prevented the development of all larvae at a concentration of 1.0% but not at a concentration of 0.5%. Thus, while Detergent A was the most effective of the 3 in clean water, Detergent B was the most effective in feces.

If one were to assume that these detergents acted primarily on the most sensitive stage, the first stage larvae, he could then estimate that Detergent A was 500 times less toxic in feces than in clean water, that Detergent B was 10 times less toxic in feces, and that Detergent C was approximately 3 times less toxic in feces. In this connection it might be mentioned that the manufacturer recommends the first detergent for use in solutions containing less than 1% acids, bases, salts, dyestuffs or

TABLE 2.—*Effect of 0.38% detergent A on horse strongyle eggs and larvae in feces*

	Days incub. at room temp.	Numbers of eggs or larvae per gram of feces			
		Unembryonated eggs	Embryonated eggs	Live larvae	Dead larvae
Treated feces	0	1500	0	0	0
	1	300	500	29	0
	2	71	57	700	0
	4	20	0	300	40
	8	0	0	100	164
Controls	0	1500	0	0	0
	1	300	1200	0	0
	2	20	40	1000	20
	4	14	0	900	100
	8	33	0	1300	0

starches, the second detergent for solutions containing 1–5% acids, bases or salts, and the third detergent for solutions containing more than 5% dissolved materials.

In order to get some idea of how these detergents act in feces, 4 experimental jars containing 0.38% of Detergent A in feces and 4 control jars were set up. At the time they were set up, the feces contained 1500 unembryonated eggs per gram. After 1, 2, 4 and 8 days, respectively, Stoll counts were made on one treated and one control jar.

The results are shown in Table 2. This concentration of the detergent is not sufficient to kill all the developmental stages, but it does kill the greater percentage of them. After 8 days, the original 1500 eggs were represented by 1300 live larvae in the control and 100 live larvae in the detergent-treated feces. There was no significant difference at any time in numbers of unembryonated eggs between the treated and control feces, or in numbers of embryonated eggs on any day except the first; after 1 day's incubation there were 500 embryonated eggs per gram in the treated feces and 1200 per gram in the control. At the same time there were 300 unembryonated eggs per gram in both jars. The peak of live larvae in the treated jar (700 per gram) was reached after 2 days' incubation, at which time there were 900 per gram in the control. Thereafter the number of live larvae in the treated feces dropped off, while that in the control remained about the same or increased. This is too small an experiment on which to base many quantitative conclusions, but it appears that the detergent acted on both the larvae and eggs. The swarm of bacteria in feces easily explains why relatively few dead larvae or eggs were observed.

DISCUSSION

Jaskoski (1950, 1951) has studied the effects of detergents on the eggs of the common swine roundworm, *Ascaris lumbricoides*. He found that 5.0% Duponol 80 (active ingredient, sodium octyl sulfate), Aresklene 400 (active ingredient, disodium dibutyl-*o*-phenylphenoldisulfonate), Areskap 100 (active ingredient, sodium butyl-*o*-phenylphenolsulfonate) and Aresket 375 (active ingredient, sodium butylbiphenylsulfonate) inhibited cleavage. In further studies, Jaskoski (1952) reported on the effects on *A. lumbricoides* eggs of Tergitol 08 (active ingredient, sodium octyl sulfate), Compound 1664 (active ingredient, sodium nonadecyl sulfate), Duponol 80, Product BC (active ingredient, *c*-betyl cetaine), and Sterox SK (active ingredient, polyoxyethylene ester). Of these detergents, Duponol 80 was by far the most effective in preventing cleavage. At 38° C., cleavage was prevented in normal eggs by 6 hours' exposure to 5% Duponol 80, and cleavage of "decoated" eggs (from which the protein outer shell layer had been removed by treament with antiformin) was prevented by 7 hours' exposure.

Jaskoski (1952) also studied the effects of these detergents on the hatching of the eggs after they had been allowed to incubate for at least 2 weeks prior to treatment. No normal eggs hatched when transferred to the detergent solutions, but hatching took place among the decoated eggs. The most effective detergent was 5% Duponol 80, in which all the eggs hatched in 3 hours at 38° C. In 5% Product BC, 80% of the eggs hatched in 8 hours. Hatching also took place in 100% Duponol 80 and Product BC. No eggs hatched in the other 3 detergents. Jaskoski does not mention the effects of the detergents on the hatched larvae. A comparison of Jaskoski's

findings with ours indicates that *A. lumbricoides* eggs are much more resistant to detergents than are horse strongyle eggs. It is of interest that in his work the detergents caused decoated *Ascaris* eggs to hatch, while in ours they prevented normal strongyle eggs from doing so.

SUMMARY

Three detergents whose surface-active ingredients are alkyl sodium sulfates were toxic for washed horse strongyle eggs and larvae in water. Embryonation of eggs was prevented by 0.05% of a detergent whose surface-active ingredient was the sodium sulfate derivative of 3,9-diethyltridecanol-6. In a concentration of 0.025%, this detergent prevented embryonated eggs from hatching; 0.01% killed all first stage larvae, 0.025% killed all second stage larvae, and 0.1% killed 94% of third stage larvae in less than a day. Embryonation of eggs was prevented by 0.6% of a detergent whose surface-active ingredient was the sodium sulfate derivative of 7-ethyl-2-methylundecanol-4, while 0.5% of this detergent prevented embryonated eggs from hatching, 0.2% killed all first stage larvae and 93% of second stage larvae, and 0.6% killed 95% of third stage larvae in less than a day. Embryonation of eggs was prevented by 1.5% of a detergent whose surface-active ingredient was the sodium sulfate of 2-ethylhexanol, while 1.0% prevented 90% of the embryonated eggs from hatching, 0.8% killed 94% of first stage larvae, 1.5% killed all second stage larvae, and 4.0% killed 94% of third stage larvae in less than a day. In general, somewhat lower concentrations were effective after 4 days' exposure.

In horse feces, it required 2.5% of the 3,9-diethyltridecanol-6 and 2-ethylhexanol detergents or 1.0% of the 7-ethyl-2-methylundecanol-4 detergent to kill all larvae or eggs.

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MAINTENANCE OF *SCHISTOSOMATIUM DOUTHITTI* (CORT, 1914) IN THE LABORATORY (TREMATODA: SCHISTOSOMATIDAE)[1]

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INTRODUCTION

In the United States, in addition to several avian schistosomes and *Heterobilharzia americana* found in the raccoon and lynx (Price, 1943), we have *Schistosomatium douthitti* occurring naturally in muskrats and field mice. *S. douthitti* is readily maintained in the laboratory and its life cycle is essentially the same as the species parasitizing man. In the present paper methods which have proved successful in the maintenance of *S. douthitti* in the laboratory and the results obtained are recorded.

Research with *S. douthitti* is adaptable to both the small and large laboratory. Since this parasite is not pathogenic for man (except for dermatitis) but produces typical pathology in laboratory animals it is excellent for teaching and demonstration purposes. Moreover, working with *S. douthitti* does not come under the jurisdiction of the federal quarantine laws and the precautions that must be employed with imported pathogenic material need not be observed.

The work herein reported was initiated in the laboratory of Dr. George R. La Rue at the University of Michigan. The data and observations are based on several years of independent research in Ann Arbor, Michigan (RBS), Chicago, Illinois and Philadelphia, Pennsylvania (IGK) and over one and one-half year's cooperative work in Tallahassee, Florida (RBS and MMN). Many of the methods outlined in the following paper were not developed by us. We have received much valuable advice and help from various workers interested in schistosomiasis. Visits to the laboratories of Dr. Eloise B. Cram and Mr. Nathan DeWitt, Laboratory of Tropical Diseases, National Institutes of Health, and Dr. Donald V. Moore, Department of Preventive Medicine, New York University Medical School have been most helpful.

HISTORICAL REVIEW

The first life cycle in the genus *Schistosomatium* was reported by Tanabe (1923a) for *S. pathlocopticum,* which he described as new using material recovered from white mice experimentally infected with cercariae from *Lymnaea palustris* (Müller), collected in or near Boston, Massachusetts. Price (1931) in Ann Arbor, Michigan, described the complete life history of *S. douthitti,* presenting detailed

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morphological studies on the various stages. A comparative study by Penner (1942) of *S. pathlocopticum* and *S. douthitti* led him to synonymize *S. pathlocopticum* with *S. douthitti.*

The cercaria of *S. douthitti* was described by Cort (1914, 1915) from infected *Lymnaea reflexa* Say collected near Chicago, Illinois. Natural infections with *S. douthitti* have since been reported from the Douglas Lake region, Michigan in *Lymnaea stagnalis appressa* Say, *L. stagnalis perampla* Walker by Cort (1917); in *Physa ancillaria parkeri* Currier by Cort (1918); in *Stagnicola exilis* (Lea), *S. palustris elodes* (Say) (= *Lymnaea palustris*), and *S. emarginata angulata* (Sowerby) by Cort (1936a). In addition, Brackett (1940) reported as snail hosts in Wisconsin: *Lymnaea stagnalis lillianae* F. C. Baker; *L. stagnalis jugularis* Say; and *L. stagnalis sanctaemariae* Walker; and Price (1931) reported *L. stagnalis appressa, L. palustris* (Müller); and *Physa gyrina elliptica* Lea as hosts near Ann Arbor, Michigan. If Penner's conclusion is accepted that *S. pathlocopticum* is a synonym of *S. douthitti,* Tanabe's (1923a) finding of *L. palustris* infected with this parasite in Boston, Mass., should also be added.

Studies on germinal development in the mother and daughter sporocysts of *S. douthitti* were made by Cort, Ameel and Olivier (1944). Recently their work on the daughter sporocysts was critically re-examined by Cort, Ameel and Van der Woude (1953). The results of these studies suggest that the method of multiplication of the intra-molluscan phase of *S. douthitti* fits the germinal lineage hypothesis with extended polyembryony.

The biology of *S. douthitti* cercariae was studied by Cort and Talbot (1936). It was shown that cercariae emerge soon after dark and after a brief free swimming period adhere to the surface film of the water by their acetabula. Olivier (1951) reported on the influence of light on the emergence of cercariae and was able to reverse the shedding cycle by placing snails in the dark during the day and under light at night. Dermatitis in man has been experimentally produced by *S. douthitti* cercariae (Cort, 1928, 1936a) and this species has been strongly suspected of causing some cases of dermatitis in persons wading in swampy areas (Cort, 1936b). Cort (1950) recently reviewed the literature on schistosome dermatitis and discussed the role of *S. douthitti,* among others, as a dermatitis producer. Infections in snails with single miracidia of *S. douthitti* were produced by Short (1952a) and the sex of the cercariae was determined. In agreement with results on other schistosomes, all cercariae from a single miracidium produced worms of one sex.

Natural infections of *S. douthitti* were found in two *Microtus p. pennsylvanicus* by Price (1931). She experimentally infected *Peromyscus maniculatus, Mus musculus,* rat and cat. She found that eggs were not passed in the feces of the rat and worms did not develop to complete sexual maturity in the cat. In addition to these hosts, Penner (1939) experimentally infected the muskrat, guinea pig, and snowshoe hare. His recovery of naturally infected muskrats from the vicinity of Minneapolis, Minnesota (32 of 330 examined) and the University of Michigan Biological Station (1 of 2 examined) led him to believe that young muskrats are the natural definitive hosts (Penner, 1938). Penner (1941) exposed a young rhesus monkey to thousands of cercariae and recovered schistosomulae in the lungs 5½ days later. Kagan (1953) recovered sexually mature worms in the portal system of rhesus monkeys necropsied 12–25 days after exposure. Infections in rhesus monkeys spontaneously terminated in this host 2–3 weeks after exposure.

The early morphological development of *S. douthitti* in the definitive host was studied by El-Gindy (1951), who gave special attention to the development of the sex organs. He reported that some worms may mature sexually as early as 10 days after infection. El-Gindy (1950) also investigated the route of migration from skin to portal system in mice, and contended that the young worms migrate from the lungs to the liver through the pleural cavity. Short (1952a) reported on the development of worms in mice with one and two sex infections. He found that both sexes mature regardless of the presence of the other, and maturity is attained in 12–13 days in both types of infection. Moreover, the eggs produced by female worms in unisexual infections develop parthenogenetically. Snail infections and resulting progeny of such uniparental miracidia were later reported (Short, 1952b). The same author also reported instances of female specimens with immature testes (1951). Short (1948), in further experiments with intergeneric crosses between *S. douthitti* and *S. mansoni,* found that female *S. mansoni* when with male *S. douthitti* developed to sexual maturity. Olivier (1952) studied the early pathology in mice exposed to *S. douthitti, S. mansoni* and *S. japonicum.* He found similarities in the pathology of the lungs caused by *S. douthitti* and *S. japonicum.*

Immunological studies have been made by a few investigators. Tanabe (1923b) studied the complement fixation test with *S. douthitti* and its group reaction with *S. japonicum.* Culbertson (1936) found that the cercariae of *S. douthitti* were resistant to the sera of many mammalian species but were killed by the sera of water snakes and green frogs. Kagan (1952) demonstrated that mice infected with *S. douthitti* develop an acquired immunity against superinfection. In a second study, Kagan and Lee (1953) found that approximately 2–3 weeks after effective chemotherapy, acquired immunity in mice was lost. Olivier (1949) demonstrated that schistosome dermatitis is a sensitization phenomenon and was able to sensitize human volunteers with cercariae of *S. douthitti* as well as with those of avian schistosomes. Immunological experiments with monkeys initially exposed to infections with *S. douthitti* and challenged with infections of *S. douthitti* and *S. mansoni* were made by Kagan (1953). He showed that monkeys were immune to the homologous species but not to the heterologous species. El-Gindy (1950) conducted experiments on immunity to reinfection in snails infected with *S. douthitti.* From his experiment he concluded that snails were immune to reinfection. Nolf and Cort (1933) reported that *L. stagnalis* infected with *S. douthitti* exhibited a partial, nonspecific immunity against the penetration and encystment of a strigeid cercaria.

Comparative chemotherapeutic studies of four antimonial compounds and Miracil D (1-diethylaminoethylamino-4-methylxanthone-hydrochloride) were made by Kagan and Lee (1952) with mice infected with *S. douthitti, S. mansoni* and *S. japonicum.* In this work, infections with *S. douthitti* were refractive to Miracil D, but responded to treatment with antimonials.

Porter (1938) reported *S. pathlocopticum* from an experimental infection in South Africa; but Penner (1942) pointed out the possibility that she was dealing with another species in the genus. A brevifurcate apharyngeal distome cercaria described as *Cercaria jaenschi* from *Ameria pyramida* and believed to be closely related to *S. douthitti* was described from Australia by Johnston and Cleland (1937).

SNAIL HOST

The snails used in our experiments are of two species, *Lymnaea stagnalis appressa* Say and *L. palustris* (Müller). Due to easier availability, *L. palustris* has been used more extensively and is being used exclusively at present. The original stock of *L. stagnalis* was obtained from Cheboygan Co., Michigan and of *L. palustris* from areas around Ann Arbor, Washtenaw Co., Michigan.

Snails infected with *S. douthitti* were collected for our work in several localities in both Michigan and Illinois. Near Ann Arbor, Michigan a collection of 665 *L. palustris* yielded 22 natural infections with *S. douthitti* (3%). Collections made by El-Gindy (1950) yielded 50 infections in 700 snails (7%). Near Chicago, Illinois, 50 infected snails were recovered in a collection of 600–800 *L. palustris* (6–8%) from small isolated ponds with large muskrat colonies. *S. douthitti* is probably endemic in many areas of the United States. Favorable places to seek infected snails are small pools or ditches near woods or isolated ponds with muskrat houses.

The methods of maintaining snails in our laboratories are similar to those used by other investigators (Noland and Carriker, 1946). Snails have been reared routinely in two types of containers: 1) glass aquaria, usually of 5 or 10 gallon capacity, and 2) shallow, white enameled pans (20 in. × 12 in. × 4 in.) with a capacity of 1½ gallons. A brief description of the methods employed follows.

Aquaria are started with 1 to 2 inches of thoroughly washed sand or fine gravel and aquarium plants. The presence of plants is not entirely necessary in an aquarium as is demonstrated by rearing snails for various periods without plants. The chief function of the plants seems to be furnishing places for attachment of egg masses and sometimes serving as a source of food. Water from the following sources proved satisfactory for rearing snail colonies: clear spring water; city tap water, which had been filtered through an activated charcoal filter to remove all traces of chlorine or allowed to stand in a large tank for several days or in carboys that were aerated; river water from the Wakulea River in Florida which is exceptionally clean and of constant chemical composition throughout the year.

Ordinary sticks of blackboard chalk or powdered calcium carbonate are supplied to the aquarium to provide material for the snails' shells. In addition, "vita-min" bricks, a commercial aquarium product, are added. These are said to contain calcium, phosphorus, iron, iodine, copper, zinc, manganese, magnesium and vitamins A, B, B_2, C, D, and G. They are recommended for aquaria with fishes, and apparently aid in the growth of snails, although controlled experiments in our laboratories have not been conducted to determine the extent of their value.

Food for the snails consisted of fresh, washed, green leaves of head lettuce, supplied every few days as needed. Rotting pieces of lettuce should be removed to avoid contamination of the water. Dried maple leaves are also excellent food; the leaves are soaked for a few weeks in several changes of water and then added to the aquaria. In addition, the water plants are eaten by the snails and must be replenished periodically.

The aquaria are aerated, either from compressed air outlets or by means of small electric aerators. Air is broken into fine bubbles in the aquaria by use of air stones attached to rubber or plastic tubing. Water in the aquaria is usually changed

every two to three months, at which time the sand is washed and the aquaria set up again as originally.

When pans are used, into each pan is put water, a small handful of gravel, dried maple leaves and a "vita-min" brick. In addition to the leaves, snails are fed lettuce or cabbage twice a week. The water in the pans is aerated and, unless the water becomes foul, snails do not climb out.

In addition to aquaria and pans, fingerbowls and small glass jars are sometimes used for maintaining snails in isolation for varying periods. The water in these containers is changed every day or two and fresh lettuce or dried maple leaves supplied as needed. Sometimes a small piece of chalk is also placed in each fingerbowl when the period of isolation lasts a week or longer.

The snails have been maintained at ordinary room temperatures, except in Tallahassee, Florida where they were reared in an air-conditioned room. The temperature in this room remained about 22–24° C., occasionally dropping to 21° C. or rising to 26° C. for short periods. A few snails have been reared at room temperature in Tallahassee and were able to withstand the prolonged hot summers of that area.

At temperatures of 22–24° C. stocks of *L. palustris* have grown well and produced egg masses practically the year round. Egg masses are usually attached to water plants or the dead maple leaves; sometimes the masses are also found on the sides of the aquaria. No special effort has been made to determine the minimum age at which egg-production begins under these conditions. It has been noted, however, that some infected snails deposited egg masses, with eggs which hatched, in isolation jars at 68–74 days of age (all snail ages computed from date of hatching).

Snails exposed to miracidia are usually young specimens. Young snails, of known ages, are obtained by removing egg masses from the stock aquaria to fingerbowls in about 150 cc. of water. Masses may be removed from the sides of aquaria by careful use of a razor blade. The water in these fingerbowls is changed every 2 or 3 days and records on time of hatching are kept. As commonly observed, hatching of all individuals in one egg mass does not occur simultaneously, but may extend over a period of 3 or 4 days. The young snails apparently derive some nourishment from the algae and bacterial growth on the plants and sides of the containers, and, in addition, tender lettuce leaves are added for food. The lettuce is apparently eaten more rapidly by these young snails if it is made tender by boiling about 1 or 2 minutes.

In stock aquaria, maintained chiefly as a source of egg masses, snails are removed occasionally to prevent excessive overcrowding; but generally no special effort is made to limit the number of snails. Snails exposed to miracidia are handled more carefully. It has been found that 20–25 young exposed snails will live and grow well in a 5 gallon aquarium, while about twice that number do well in a 10 gallon tank. At the end of the incubation period, the snails are isolated and examined for cercarial production. Only the infected snails are returned to aquaria, leaving usually, fewer snails per aquarium than originally. Young progeny of the infected snails are removed periodically to avoid crowding and confusion with infected specimens.

Infected snails for which longevity records were kept were maintained for most

of their lives in relatively uncrowded conditions, with 2 to 13 snails per aquarium. Some of these snails have grown to a fairly large size, the largest of any in our laboratories. The largest of these specimens have shell lengths of 32 to 33 mm., sizes which compared favorably with those of the largest snails of this species in some natural populations.

Both infected and unifected snails have been successfully shipped hundreds of miles within the country and to France and Germany. Snails were placed in small perforated metal mailing containers surrounded by damp sphagnum and shipped air mail express. Large numbers of mature and young snails withstood periods of 5–7 days in transit and arrived alive.

The longevity of laboratory-reared snails is of some importance, especially when the snails are infected and it is necessary to have available a constant supply of cercariae for experimental purposes. Three snails with single miracidial infections lived for periods of 361, 635 and 687 days respectively after exposure (Short, 1952a). More recently additional data on longevity of infected snails have been

TABLE 1.—*Longevity of* Lymnaea palustris *exposed to 3–5 miracidia of* Schistosomatium douthitti

	No. snails	Age (days)	Duration of infection (days)
Exposed and infected		Minimum Ages	
4/16/52–4/27/52	61	29	...
Alive and shedding cercariae			
4/28/53	27	382	365
5/28/53	18	412	395
6/11/53	12	425	408
8/ 6/53	8	468	451
8/22/53	7	486	469
9/ 4/53	3	499	482
10/ 2/53	2	527	510
10/ 7/53	1	532	515
10/24/53	0	549	532
Exposed and infected			
9/25/52	12	149	...
Alive and shedding cercariae			
1/13/53	12	259	110
4/29/53	5	365	216
6/11/53	4	408	259
6/25/53	3	422	273
7/23/53	2	450	301
8/ 6/53	1	464	315
8/20/53	0	479	330

gathered from several lots of snails, exposed in small groups to several miracidia per snail. The data on 2 groups are presented in Table 1. One lot of 61 snails, 17–29 days old at time of exposure, were reared in the air-conditioned room (see above). The second group of snails were 149 days old at exposure and were maintained at room temperature. From the data in Table 1 it will be noted that under proper culture conditions, about half the infected snails may live to an age of 1 year; and that the duration of infection depends on the age of the snail when exposed. A few snails infected when young may carry infections for as long as 16–18 months. Of importance is the fact that snails up to 149 days, and probably older, can be readily infected. It is however usually desirable to expose young snails in order to obtain longer periods of cercarial production.

Miracidia for snail exposures may be obtained by hatching eggs present in host's feces or in liver tissue by using the side arm flask method described by McMullen and Beaver (1945) for bird schistosomes. This type of 1 liter flask is delineated

in Figure A. Our data do not indicate whether there is a difference in infectivity or viability of eggs hatched from feces or from the liver. Eggs obtained from both sources have given satisfactory results, but livers are usually used when large numbers of miracidia are desired. Water of the same types as used for rearing snails has proven satisfactory for hatching miracidia and exposing snails.

The methods used for obtaining miracidia from eggs in liver tissue follow the general techniques described by McMullen and Beaver (1945) for feces, with the following modifications: livers are torn into small pieces and then ground with a small amount of wet, coarse, clean sand, using a mortar and pestle or placed whole in a semi-micro Waring blender cup in a small amount of fluid and blended for 20 seconds. The ground or blended tissue may be directly transferred to the side arm flask with water or washed in several changes of 0.85% saline by decantation in a 500 cc. pharmaceutical graduate cylinder tapered at the bottom. Eggs of *S. douthitti* are inhibited from hatching by 0.85% saline. After the supernatant is clear, usually follow three 20 minute changes, the saline is carefully decanted and the material containing the eggs of *S. douthitti* transferred to the side arm flask with fresh water. After transfer is made the flask is filled first to the level of the dotted line (Fig. A), then to the brim, by rapidly pouring more water into the side arm. This procedure leaves clear water in the side arm. A paper bag with a hole of suitable size and position is then placed over the flask so that the arm protrudes, but the rest of the flask is covered. The flask is then placed 12–15 inches from a light source.

Miracidia, which are negatively geotropic and positively phototropic, swim into the clear water of the side arm, where they can be seen against a dark background with the naked eye. The first miracidia usually appear after about 5 to 10 minutes. Several hundreds or thousands of miracidia may hatch from a single liver and be swarming in the side arm within an hour after the flask has been filled and may be removed from the side arm with a pipette. After several hours, miracidia may cease to appear. Another group may sometimes be hatched by replacing most of the water in the flask. Water originally put in the flask is carefully siphoned off to a depth of about 1½ inches. A little water is added; the flask is vigorously shaken, then filled with water as originally. Placing the flask in a refrigerator overnight and replacing the water with water at room temperature the following morning will induce many new miracidia to hatch. This technique once yielded hundreds of miracidia per day over an 11 day period.

Snails have been exposed in groups to several miracidia per snail, individually to several miracidia, and to one miracidium each. Groups of snails were exposed in fingerbowls, stender dishes or occasionally aquaria. Snails were individually exposed most successfully to one or several miracidia in small stender dishes, vials or glass cups about 10 mm. diameter by 10 mm. deep. The small glass cups are handled more readily when several are cemented to small rectangular glass plates. Miracidia placed in these small cups may be observed under the binocular microscope and counted. After the miracidia and snails have been introduced, the containers should be covered to prevent escape of snails and evaporation of water. The snails are removed from the exposure containers after about 20–24 hours and reared in aquaria or pans until time for cercarial emergence.

Snails exposed to miracidia in the laboratory have been, with few exceptions, laboratory reared. In our work no special study has been made of variations to susceptibility of snails at different ages. Generally the snails exposed have been rather young, about 3 to 10 weeks old. However, the data of Table 1 show that young snails and considerably older snails were readily infected.

Data on the exposure of 1764 young snails of undetermined age reared in the laboratory in Michigan, Illinois and Pennsylvania over a 2 year period between March 1950 and December 1952 are presented in Table 2. Although these snails were of unknown age they were generally selected because of their small size and probably were 1 to 4 months old. Each snail was exposed individually in a small glass cup to several miracidia (generally not exceeding 10). The percentage of snails that became infected after exposure in the Florida series was significantly higher. Of interest is the fact that the snails used in the Florida work are the progeny of stocks sent from the Illinois laboratory. In the Florida series, snails ranging in age from 16 to 18 days to 149 days were exposed. There is no apparent significant variation in susceptibility of these different age groups. For example, in a group of 31 snails, 23–25 days old 19 were recovered after exposure and all

TABLE 2.—*Exposure of* Lymnaea palustris *and* L. stagnalis appressa *to several (less than 10) miracidia of* Schistosomatium douthitti

No. of snails exposed	No. of snails recovered	% survived	No. of snails infected	% snails infected[1]
1764	1252	71	293	23.4
381[2]	299	78	250	83.6

[1] Percentages calculated on the number of snails recovered.
[2] *L. palustris* maintained in air conditioned room in Tallahassee, Florida; exposed to 3–5 miracidia each.

were infected. In another group of 15 snails 149 days old, 12 were recovered and all were infected. The rate of infection for all 15 lots ranged from 73.7% to 100%. Although all snails were exposed in the air conditioned room, 41 snails in 2 aquaria were maintained subsequently at room temperature. The percentages infected in these 2 tanks were 80% and 92.3%. It is evident, then, that the parasite developed as well in snails maintained at room temperature as in those reared in the air-conditioned laboratory.

Individual lots of snails exposed in Illinois yielded 50–80% infection. These snails were generally exposed during the spring of the year or early summer. During a winter period between December 18, 1950 and April 6, 1951, in Illinois, 380 snails in 16 lots averaged an infection rate of 5.5%. Although snails were exposed in warm rooms they were reared in rooms that were not well heated and the temperatures may have been below the minimum required for cercarial development.

The incubation periods were determined for 116 laboratory-reared *L. palustris,* infected with one miracidium each at 23–35 days of age and reared in an air-conditioned room. Snails were isolated daily before dark and examined the following day for cercariae. As can be seen in Figure B, incubation periods varied from 37–52 days after exposure, with the largest numbers of infections maturing at 42–44 days. Measurements of lengths of snail shells on day of first shedding average 14.2 mm., with a range of 5–23 mm. The mean number of cercariae shed on first day was 9.8 with a range of 1–55.

TABLE 3.—*Sex of cercariae obtained from snails, each exposed in the laboratory to several miracidia (less than 10) and snails naturally infected with* Schistosomatium douthitti

	No. of snails tested	Male-female infections	Female infections	Male infections
Laboratory infected snails	113	36 (31.8%)	26 (23.0%)	51 (45.1%)
Naturally infected snails	36	11 (30.5%)	10 (27.9%)	15 (41.7%)

The number of cercariae shed by an infected snail increases with time, especially during the first few days. Snails with infections over one year may produce several hundred cercariae in one evening's shedding. Snails in the laboratory do not generally produce as many cercariae as naturally infected snails. This fact may be the result of the smaller size usually attained by laboratory snails. It appears that the numbers of cercariae produced are probably positively correlated with the size of the snail when the daughter sporocysts are developing. Larger snails provide more space for development. We have also noticed what appears to be an increase in cercarial production over a period of several months. This increase appears to be positively correlated with growth of the snail after the end of the incubation period, suggesting that, even in mature infections, cercarial productivity increases as the snail grows. Some of the larger experimentally infected snails have yielded 500–1500 cercariae per day. As many as 1000–5000 cercariae per snail have been recovered from naturally infected *L. palustris* in one evening.

Observation of snails that were isolated daily for cercariae revealed that cercariae do not emerge every day. From some snails cercariae emerge for 2 to 3 days and skip a day or two. In one instance 7 infected snails yielding cercariae for several weeks ceased producing. Examination of the livers of these snails several weeks later revealed that the infections had terminated.

The sex of cercariae of 36 naturally infected snails was determined by infection of mice. In addition, 113 snails infected in the laboratory by exposures to several miracidia (Table 3) and 90 snails exposed to one miracidium each (Table 4) were tested. The percentage of infections in laboratory infected snails exposed to more than one miracidium and naturally infected snails was very similar. For snails exposed to a single miracidia the sex of cercariae infections did not deviate significantly from a 1 : 1 sex ratio.

TABLE 4.—*Infections in snails 15–150 days of age exposed to one miracidium of* Schistosomatium douthitti

Species	No. exposed	Age of snail in days	No. recovered and examined	Infected		No. for which sex of cercariae was determined	Male	Female
				No.	%			
L. stagnalis[1]	83	14–140	80	12	15.0	12	7	5
L. palustris[1]	121	14–150	110	12	10.9	12	7	5
L. palustris[2]	259	23–35	213	116	54.5	51	16	35
L. palustris	...		...	...	...	15	8	7
Totals	463		403	140		90	38	52

[1] Data from Short (1952a).
[2] Same snails as considered in Fig. B, for which incubation period was determined.

MAMMALIAN HOSTS

Mice, rats, hamsters, rabbits and monkeys have been infected with *S. douthitti* in our laboratories. The techniques for exposing these hosts varied with the purposes of the infection and were of two main types. Exposure by immersion in water containing cercariae was often used for routine maintenance of infected animals to provide eggs for obtaining miracidia. Several mice or hamsters were placed for a half hour in a battery jar or other small receptacle filled to a level of 1–1½ inches with water containing cercariae. Covered fingerbowls have been used for single mice and young hamsters and quart fruit jars proved excellent for large hamsters. Rabbits, after clipping the posterior part, have been exposed by immersion in a pail of water containing cercariae. Restraining the rabbit and immersing an ear in a finger bowl with cercariae is equally effective.

A second and more accurate method of exposure is to place cercariae directly on the host. The cercariae of *S. douthitti* after emerging from the snail cling to the surface film of the water and remain almost motionless. They can be lifted from the surface film of the water with a small loop, counted under a stereoscopic microscope and transferred to the skin of the host to be exposed. The loop is made from a strand of hair or the stylet from a 20 gauge hypodermic needle attached to the end of a wood applicator with household cement. In this manner, mice, hamsters, and monkeys have been exposed to known numbers of cercariae and quantitative data obtained.

Use of the loop technique requires that the mouse or hamster be taped to a small rectangular board (or piece of plastic 2½ inches × 6 inches for mice) or anaesthetized. The technique for taping mice down is essentially similar to that described by Olivier and Stirewalt (1952). With practice one operator can tape a mouse by holding the animal by the nape of the neck on its side against the board and passing the adhesive tape over the thorax and forelegs. The hind legs are either taped to the board or held in place with rubber bands attached to small nails fixed at the end of the board. The hair on the abdomen of the animal is clipped, the area is washed and moistened with water prior to placing the cercariae on the skin. Animals are returned to their cages ½ hour after exposure.

The use of an anaesthetic apparently does not interfere with infection. Intraperitoneal injection of sodium pentobarbital (Veterinary Nembutal, 60 mg. per cc.) has been used with success. For mice a 15% solution of pentobarbital in sterile distilled water administered intraperitoneally with a tuberculin syringe in the ratio of 1/100 cc. for each gram of body weight will anaesthetize for several hours. For hamsters a 20% solution administered in the same ratio is very effective. For rats, a 30% solution and administered in the ratio of 0.2 cc. per 100 grams body weight has been used. Monkeys have been anaesthetized with intraperitoneal injections of 1–2 cc. of undiluted pentobarbital.

Young worms begin to migrate from the liver to the mesenteric veins 10 or 11 days after infection (Short, 1952a); by the 13th day the majority are usually in the mesenteric veins, and after this age practically all worms (male–female infections) are found in the extra-hepatic portal vessels.

Sexual maturity is not attained at the same age by all worms, even in the same host. Some specimens of both sexes are usually mature at 10–12 days. Increasing numbers of worms become mature on subsequent days until the majority of both

sexes are mature by the 16th day and all (except anomalous ones) are mature at 20 days of age. The above data on sexual maturity were obtained from infections in deer mice (*Peromyscus maniculatus*) and albino mice (*Mus musculus*). Recent preliminary work using an inbred C3H strain of *Mus musculus* indicates the same pattern of development in this host.

Correlated with a difference in rate of maturity is a disparity in size and external differentiation of worms of the same age. Sexual dimorphism of some worms may be sufficiently evident at 10 days to separate sexes using a stereoscopic microscope (27 ×). Other worms of the same age or slightly older may be so undeveloped as to render sexing difficult or impossible by gross examination. By the 15th day the sex of practically all worms may be distinguished without difficulty. In deer mice, and probably in other hosts as well, maximum size of both sexes is reached at about 30–40 days.

Mature miracidia have been found in host tissues as early as 26 days after infection. We routinely obtain eggs with miracidia in feces and/or liver of hosts with infections of 40–150 days duration. The findings of Kagan and Lee (1952) suggest that miracidia of *S. douthitti* may live for only 3–4 weeks in host tissues. Animals with old infections do not produce as many hatchable eggs as hosts with infections of shorter duration.

Death of mammalian hosts is sometimes caused by infections with *S. douthitti* especially when excessive numbers of cercariae are used. Death often occurs about 3 days after exposure in very heavily infected mice (300–500 cercariae) or soon after 30 days in less heavily infected ones (100–300 cercariae). The former time coincides with the time of migration of young worms through the lungs, and death at this time apparently results from lung hemorrhages. After 30 days, death is apparently due to hemorrhage of the cecum and adjoining part of the large intestine caused by many eggs breaking through the intestinal wall. For mice, exposure to 50–75 cercariae of both sexes is recommended. For purposes of maintenance of the infection in the laboratory, hamsters of 50–80 gm. body weight should receive a similar dose, but large hamsters may receive up to 150 cercariae.

Data on longevity of adult worms demonstrate that their life span in favorable hosts can be well over one year. Female worms from unisexual infections survived for 468–484 days and both sexes lived in bisexual infections for 383 days (Short, 1952a). An infection with male worms was observed in a mouse infected for 828 days. These infections were in *Peromyscus* and were the oldest examined. Since the worms were in good condition, we may infer that they could have lived longer. Price (1931) reported a considerably shorter life span in the rat and Penner (1940) recovered a single pair *in copula* plus disintegrating worms from a rat infected for 236 days. In addition to a shorter life in the rat, our experience indicates that the worms in this host are usually smaller than those in mice. Further evidence of an immunological response of a cellular nature against infection with *S. douthitti* in the rat was the observation of several mature worms encapsulated in the lung of an infected rat. Rats, therefore, appear to be less favorable hosts for *S. douthitti* than mice; the worms in rats often dying off before the host, while in mice the life span of worms (once maturity is attained) is often, possibly in most cases, limited by the death of the host.

A number of rabbits have been exposed to the cercariae of *S. douthitti*. Infec-

tion in this host has been poor. Infections in monkeys persist for 2–3 weeks after which the worms are killed and destroyed. Specimens recovered from these animals prior to 3 weeks appear normal in all respects and after 13 days have reached sexual maturity (Kagan, 1953).

The hamster is now being used routinely in one of our laboratories for maintaining the mammalian phase of the life cycle. Livers of young hamsters exposed for over 30 days contain many eggs and make excellent preparations for obtaining miracidia with the side arm flask technique. Hamsters are very susceptible hosts. Infected animals are stunted in growth and show evidence of much pathology at necropsy.

Data on the susceptibility of various strains of mice exposed in our laboratories are given in Table 5. Mice exposed in Tallahassee, Florida, proved to be slightly more susceptible than the average for the series. Methods of infecting mice in this series differed in one respect from the described technique, in that the abdomen of the mouse was kept moist for the entire period of exposure with a fine spray of water. The mouse is very susceptible to infection. In approximately 1000 necropsies only one animal was found to be negative. The data in Table 5 are not considered

TABLE 5.—*Susceptibility of mice exposed by the loop technique to varying numbers of cercariae of* Schistosomatium douthitti

Mouse species	No. cercariae per mouse	No. mice	Total no. cercariae	Total no. worms	Average no. worms per mouse	% cercariae developing
CF1	10	9	90	41	4.55	45.55
CF1	25	93	2,325	829	8.91	35.65
CF1	50	313	15,650	7,644	24.42	48.84
C3H	50 & 60	30	1,630	942	31.36	57.79
White	60–80	11	745	438	39.81	58.79
CF1	100	14	1,400	767	54.78	54.78
dba	50 & 100	9	553	360	40.00	65.09
Totals		479	22,393	11,021	....	49.21

adequate for determining whether different inbred lines of *Mus musculus* vary in susceptibility to *S. douthitti.*

Infections of hosts may be standardized and very uniform worm counts obtained if an inbred line of mice are used and if cercariae are from a common pool of infected snails. Inbred lines of mice have yielded more uniform infections probably because of their genetic homogeneity. The studies of Evans and Stirewalt (1951) demonstrated the importance of using a common pool of cercariae of *S. mansoni* for purposes of infection to eliminate the element of variation in infectivity of cercariae.

As a general rule in our work snails were isolated late in the afternoon and cercariae used the same night several hours after emergence. Although studies on length of infective life of cercariae have not been made, cercariae used the following morning or early afternoon in the Florida series appear to be just as infective.

Worms may be recovered from experimental hosts by perfusing the systemic system with a solution of 0.85% sodium citrate and 0.85% saline. The technique described by Yolles, Moore, DeGiusti, Ripsom and Meleney (1947) is employed with slight modification. The mouse is etherized or killed by breaking the neck, and the skin is peeled back exposing the body wall. The body and pleural cavities are opened and the inferior vena cava between the liver and heart ligated with cotton thread and severed. The esophagus is cut and the lungs pushed aside exposing the

dorsal aorta. The carcass is then placed in a petri dish over a white enameled tray and the hepatic portal vein severed. A 20 or 22 gauge needle attached to the perfusing apparatus is inserted into the dorsal aorta and the systemic system perfused until the petri dish is almost full. The intact liver should swell considerably and blanch as the blood flows out. To insure that all worms have been washed out of the liver the needle should be inserted into the hepatic sinus and the liver further perfused. After perfusion both liver and mesenteries are removed and placed in separate petri dishes. The liver should be torn into small pieces with forceps in a small amount of saline to allow trapped worms to crawl out. A rapid method for recovery of residual worms within the liver is to place the intact organ in a semi-micro Waring blender cup, fill the well with saline and blend for 3 seconds. The liver is broken apart into small pieces and the worms released in good condition. The mesenteries are inspected under a stereo-binocular microscope or macroscopically with the aid of a binocular loupe against the white background of the enameled pan. Worms found in the mesenteric veins are removed from the blood vessels with fine curved sewing needles attached to small wooden handles. When data are desired on location of worms, the method as originally described by Yolles *et al* (1947) should be followed.

TABLE 6.—*Distribution of worm counts in 303 CF1 mice exposed to 50 cercariae each of* Schistosomatium douthitti

No. worms recovered	No. of mice
0– 5	8
6–10	18
11–15	20
16–20	43
21–25	52
26–30	62
31–35	57
36–40	29
41–45	8
46–50	6

In the absence of pressure for a perfusion apparatus, recovery of worms in mice is greatly facilitated by perfusing the animal using a 50 cc. syringe. Although this method does not remove all the worms, it dilutes the blood in the mesenteries with saline and facilitates finding and picking out the schistosomes.

The sex of 3642 worms recovered from 181 CF1 mice exposed to pooled cercariae of undetermined sex was tabulated. Male worms (55.35%) slightly outnumbered female worms. Slightly higher recovery of male worms in bisexual infections have been reported for other schistosome species, when pooled cercariae of unknown sex had been used.

The distribution of the number of worms recovered in 303 CF1 mice of both sexes exposed to 50 cercariae each is given in Table 6. In this entire lot all mice were infected; the lightest infections are represented by 6 mice, 2 each with 1, 2 and 3 worms; the heaviest infection by 1 mouse with 50 worms from 50 cercariae. The mean number of worms per mouse was 25.08 (50%).

DISCUSSION

The maintenance of *S. douthitti* in the laboratory does not present any particular problems that differ radically from the maintenance of other schistosome species. Rearing and infecting the snail host is perhaps the most difficult part of the life

cycle; however, with sufficient space and correct methods, lymnaeid snails may be reared in any quantity desired and readily infected.

FIG. A. Flask with side arm for the hatching and concentration of schistosome miracidia (from McMullen and Beaver, 1945).

In recent years there has been a considerable amount of work on the biology and maintenance of the schistosome species infecting man. Biologically *S. douthitti* has a life cycle that is similar to that of species infecting man. Our findings are in general accord with the results of other workers with *S. mansoni* or *S. japonicum.*

SUMMARY

For several years *Schistosomatium douthitti* has been maintained successfully in the laboratory. A description of the techniques used to rear snail colonies of *Lymnaea stagnalis appressa* Say and *L. palustris* (Müller) in aquaria, trays and fingerbowls is given. In addition are described: methods for obtaining miracidia from liver tissue, infecting young snails, obtaining cercariae from infected snails, exposing mice, hamsters, rabbits and monkeys to quantitative doses of cercariae, and a rapid method for the recovery of worms.

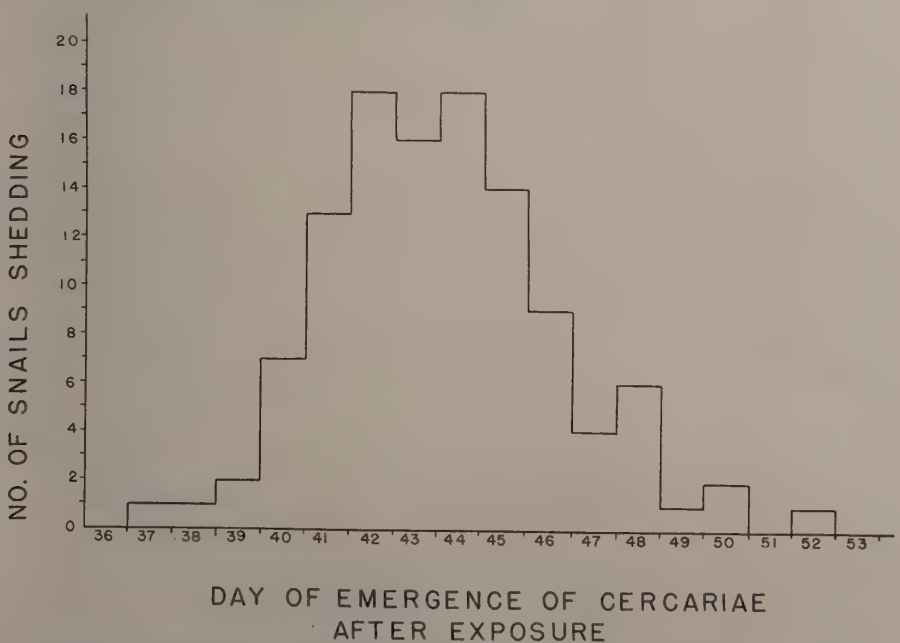

FIG. B. Lengths of incubation periods of *Schistosomatium douthitti* in *Lymnaea palustris.* Each snail was 23–25 days old when exposed to one miracidium.

Observations on the development of *S. douthitti* in the intermediate host are made. Data on the longevity of infected snails are given. The infectivity of laboratory reared snails to exposure of one and several miracidia is tabulated and the sex of cercariae for naturally infected snails, snails exposed to 5–10 miracidia and snails exposed to one miracidium each was determined by infection in mice. The incubation periods for snails infected with one miracidum each were determined.

Observation on the development of *S. douthitti* in the definitive host are made. The time required for worms to reach sexual maturity in *Peromyscus maniculatus* and albino mice was determined and data recorded on the longevity of infections. The susceptibility of various laboratory hosts are discussed and the number of worms recovered from a group of CF1 mice exposed to 50 cercariae each is tabulated.

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THE INTESTINAL HELMINTHS OF THE COYOTE *CANIS LATRANS* SAY, IN UTAH

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This study of the helminths of the coyote, *Canis latrans* Say, 1823, was initiated following the appearance in Northern Utah of 7 recent human cases of hydatid cyst infection produced by the larval form of *Echinococcus granulosus* (Batsch, 1786) Rudolphi, 1805. Carlquist and Dowell (1951) reported on 4 of these cases coming to them for surgical treatment during the interval of 1948 to 1950. Three additional cases have appeared subsequent to this, of which 2 were in children of pre-school age residing in Salt Lake City. Attempts to determine the definitive host of Echinococcus in this area led us to examine 200 dogs from Salt Lake City with negative findings (Butler and Grundmann, 1951) and to examine commercial silver fox, also with negative findings (Grundmann, Butler and Selin, 1953). The possibility that the coyote might act as the definitive host of this parasite and serve as a reservoir prompted the present study.

Cameron (1927), in a paper dealing with the human hydatid problem, reported the coyote as having been found naturally infected with *Echinococcus*. However, he failed to list the source of his information. Correspondence with workers in the field and a review of the rather extensive literature pertaining to the parasitic fauna of the wild CANIDAE of North America has failed to substantiate Cameron's report.

No previous study of the parasites of the coyote has been made in this region, and since this animal is an important predator, increased knowledge concerning its ecology is necessary to the understanding of the role it plays in the habitat, especially in regard to food habits.

MATERIALS AND METHODS

Our study is based upon helminths obtained from 75 preserved intestinal tracts (pylorus to anus) from freshly killed adult animals taken by hunters of the Predator and Rodent Control Division of the U. S. Fish and Wildlife Service in 13 counties of the State of Utah. The tract was tied off at each end at the time of removal and preserved in 10% formaldehyde. These gut specimens were labeled as to date and locality. In the laboratory, each tract was completely dissected beginning at the pyloric end. Dissections were performed in a black developing tray containing water. The intestine was looped back and forth 3 or 4 times by being attached to one end of the tray by means of dissecting hooks. As the intestine was opened a few inches at a time, it was rolled on a hemostat. All helminths found were removed and the entire mucosa examined with the aid of a 10 power hand lens. Four of each 10 inches of gut were removed and the mucosa examined under a stereobinocular microscope. Loose materials and feces were screened through a 100 mesh copper screen. Where formalin preserved material is used, this procedure yields better results than techniques commonly used with unpreserved material.

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Nematodes were cleared in lactophenol containing a small amount of concentrated picric acid to stain internal structures. Small nematodes and dissected portions of larger worms were mounted in clarite. The larger worms were stored in 70% alcohol and 10% glycerine. Each of the 1321 tapeworms recovered was stained with acid alum-carmine after being flattened between glass slides, cleared in oil of cloves and individually determined to species.

RESULTS

Dissection of the 75 intestinal tracts produced a total of 1576 helminths, 255 nematodes and 1321 tapeworms. Fifty-four animals, comprising seventy-two percent, were infected by helminths of 1 or more species. Round worms of 1 or more species were removed from 29 of the tracts, and cestodes of 1 or more species from 48. Twenty-nine coyotes possessed only 1 kind of parasite, 17 contained 2, 5 contained 3, and 3 animals contained representatives of 4 species.

Of the nematodes, *Toxascaris leonina* (Linstow, 1902) was predominant, occurring in 28 of the 75 coyotes examined, and comprised 233 of the 255 specimens recovered. *T. leonina* was first reported for the coyote by Stiles and Baker (1935). Sperry (1941) also found this species during his study of coyote food habits, but Erickson (1944) did not find a single specimen in the 65 coyotes examined by him. This species was also recovered from 7 of the 200 dogs from Salt Lake City examined by us. *T. leonina* is considered to be endemic in the wild carnivora of this region, and is also known to occur locally in the mountain lion, *Felix concolor hippolestes* Merriam. Ascarid infection is probably perpetuated in the vicinity of the dens where the soil is likely to become contaminated with eggs from infected adults and readily transferred to the young adults. Examination of 20 intestinal tracts from coyote pups, not included in the above 75, showed much higher infection than in adults and tends to confirm this observation. *Toxocara canis* Werner, 1782, the other common canine ascarid, was found but once in coyotes, although it appeared in 9 of the dogs from Salt Lake City.

Several species of Oxyuridae were recovered. Of these, *Dermatoxys veligera* (Rudolphi, 1819) has not hitherto been reported from the coyote. This nematode occurs commonly in the cecum of wild and domestic rabbits and is probably present in these animals throughout the United States. Our examination of wild cottontail rabbits of the genus Silvilagus in Utah also show this parasite to be quite prevalent and widely distributed in the State. A second species of Oxyurid, *Passalurus nonannulatus* Skinker, 1931 was also recovered. This species, also a parasite of wild rabbits, was described from *Lepus americanus* by Skinker (1931) who further reported it from the coyote *Canis lestes* (= *C. latrans lestes*). *P. nonannulatus* appears to be predominantly a parasite of the cecum of *Lepus californicus deserticola* Mearns, the jack rabbit, in this region. Considerable host specificity appears to be present in the case of these two roundworms with rare individuals of *Dermatoxys* being recovered from jack rabbits. Members of the two afore-mentioned species were recovered from the same intestinal tract and are considered as cases of spurious parasitism in the coyote.

Specimens of *Protospirura numidica* Seurat, 1914 were recovered in one instance during this study. Sperry (1941) in his study of the food habits of the coyote was the first to report *P. numidica* from coyotes collected in 6 western states. It is inter-

esting that this species appeared in the most heavily parasitized intestinal tract containing 217 helminths comprising 4 species. In addition to *P. numidica,* this coyote contained specimens of *Toxascaris leonina,* and of 2 tapeworms, *Mesocestoides kirbyi* and *Taenia pisiformis.*

Seven species of tapeworms were recovered during this study, the intermediate hosts of which reflect, to a degree, the food habits of the coyote in this region and the relative availability of these intermediate hosts as food. *Taenia pisiformis* Bloch, 1780 was the most prevalent cestode encountered both in the total number of worms recovered, and in the number and distribution of animals infected. A total of 54.6% of the tracts examined showed worms of this species. This may be contrasted with the study of dogs from Salt Lake City in which 1% of the dogs possessed worms of this species. The jack rabbit, *Lepus californicus deserticola,* has been found to be an intermediate host.

Three additional species of Taenia were recovered, viz. *T. rileyi* Loewen, 1929, *T. krabbei* Moniez, 1879 and *T. hydatigena* Pallas, 1766. *T. rileyi,* first reported for *C. latrans* by Erickson (1944), was recovered from 2 tracts. The intermediate host of this tapeworm is unknown, but is possibly a mouse. One coyote contained adults of *T. krabbei,* a species first reported from the coyote by Erickson (1944). Shaw, Simms and Muth (1934) recovered the cysticercus of this species from deer in Oregon and confirmed the determination by rearing 55 adults in the bobcat, *Lynx rufus.* Wardle and McLeod (1952) found the cysticercus in reindeer in Alaska. Another species common to CANIDAE, *T. hydatigena* was taken from 2 intestinal tracts. This species was first reported from *C. latrans* by Erickson (1944). The cysticerci of this tapeworm may be found in the abdominal cavities of sheep, cattle, goats, deer, pigs and squirrels throughout the United States.

Four coyotes contained *Multiceps packii* Christenson, 1929. Parasitism of the coyotes by this species was also first reported by Erickson (1944). The worm was described by Christenson (1929) who found the coenurus at the tip of the heart of the varying hare, *Lepus americanus phaenotus* Allen from Minnesota. The intermediate host in Utah is probably one of the rabbits.

Mesocestoides kirbyi Chandler, 1944 was recovered from 8 coyotes and may be considered to be the second most prevalent species in the coyotes of this region. Its life cycle has not yet been definitely determined although the *Tetrathyridium* (= *Cysticercus dithyridium* of Villot, 1883), commonly found in reptiles and birds has long been considered to be the larval stage. *M. kirbyi* was described from several lots of *Mesocestoides* collected from *C. latrans* in California by Chandler (1944). A new record for the coyote was obtained by the recovery of the common double-pored dog tapeworm, *Dipylidium caninum* (Linn., 1758) Leuckart, 1863 from a single coyote intestine.

The species of taenioid tapeworms present in the intestinal tract and the relative abundance of each can possibly be used as an indication of the proportion of the various prey species and carrion in the diet of the coyote. The 54.6 percent that possessed *Taenia pisiformis* demonstrates that rabbits make up a considerable portion of the diet and that they are widely parasitized. The 5.3 percent of infection of coyotes by *Multiceps packii* would indicate that these forms are less prevalent. Since the larval forms of *T. hydatigena* and *T. krabbei* are known to occur in cattle, sheep and wild ruminants such as deer, some food of this type must be taken by coyotes, probably in about the same proportion of diet as the percent of infection.

SUMMARY

The intestinal tracts of 75 adult coyotes, collected from 13 counties of Utah during the period of 1949 to 1952 were examined for helminths. The tracts were taken from freshly killed animals and preserved in the field in 10% formaldehyde.

Helminths of one or more species were recovered from 54 of the 75 animals. A total of 255 nematode specimens, comprising 5 species, were found. Of these, *Toxascaris leonina* Linstow was predominant in numbers. Others present were *Toxocara canis* Werner, *Protospirura numidica* Seurat, *Passalurus nonannulatus* Skinker and *Dermatoxys veligera* Rudolphi. The last named constitutes a new record for the coyote.

Seven species of tapeworms were found comprising 1321 specimens. *Taenia pisiformis* Bloch was most prevalent. Remaining forms were *Taenia rileyi* Loewen, *T. krabbei* Moniez, *T. hydatigena* Pallas, *Multiceps packii* Christenson, *Mesocestoides kirbyi* Chandler and *Dipylidium caninum* Linn. *D. caninum* is new to the coyote.

Although a search for the definitive host of *Echinococcus granulosus* (Batsch) prompted the study, this species was not recovered.

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A NEW STRIGEID TREMATODE FROM THE PECTORAL SANDPIPER

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On May 2, 1942, 700 trematodes were collected by the second author near Kemah, Texas, from the intestine of a single adult Pectoral Sandpiper, *Erolia melanotos*. There were no observable pathogenic effects due to the infection. Most of the trematodes were flattened, fixed in Bouin's fluid, stained with carmalum, cleared in cedar oil, and mounted in balsam, but 2 specimens were serially sectioned. Thirty-six whole mounts were studied thoroughly, although the measurements given below are based on only 10 whole mounts and the serial sections. Measurements in parentheses are averages from 10 specimens. All measurements are in millimeters.

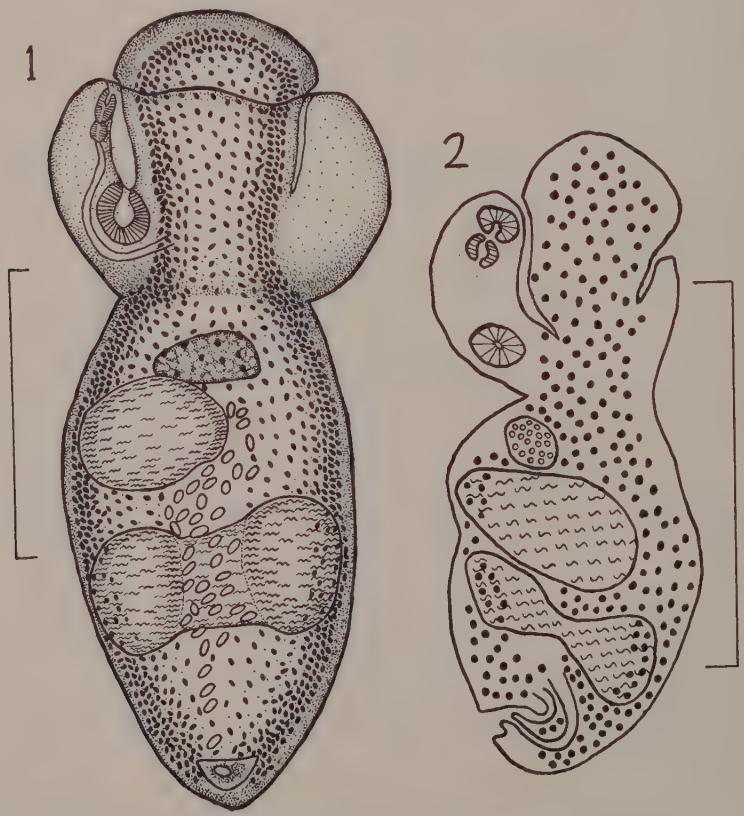

FIG. 1. Whole mount of *Strigea eroliae*, n. sp. Note the position and size of the holdfast organ. There is an egg in the genital atrium. As with most of our specimens, the worm is twisted so that the forebody is in lateral view, the hindbody in dorsal view. Scale 1 mm.

FIG. 2. Sagittal section of *S. eroliae* through anterior sucker, pharynx, and acetabulum reconstructed from three serial sections. Note the vitellaria in the extreme posterior end of the body. Scale 1 mm.

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Strigea eroliae, n. sp.

Diagnosis: Strigeidae: Forebody thick, cup-shaped, measuring 0.654 to 0.892 long and 0.714 to 0.999 wide (0.713 × 0.926). Holdfast organ thick, cup-like, protruding anteriorly from open end of forebody. Intestinal ceca twining through forebody and through hindbody almost to genital atrium. Hindbody broadly ovoid, ranging in size from 1.463 × 1.844 to 1.142 × 0.749 (1.315 × 0.957). Ratio of the length of the hindbody to that of the forebody 1.8 : 1. Oral sucker spherical, 0.083 in diameter. Pharynx small, spherical, 0.071 in diameter. Ventral sucker distinguishable in four specimens, where it measures 0.154 to 0.178 × 0.107 to 0.154 (0.162 × 0.127).

Testes large, filling most of the hindbody, posterior testis the larger, ranging in size from 0.654 × 0.357 to 0.916 × 0.476 (0.776 × 0.448). Anterior testis measuring from 0.392 × 0.273 to 0.714 × 0416 (0.491 × 0.373). Posterior testis apparently bilobed; anterior ovoid or ellipsoid in all specimens examined. Ovary ellipsoidal, 0.130 to 0.178 × 0.249 to 0.321 (0.312 × 0.162) and located anterior to testes near union of two body segments. Vitellaria extending into extreme anterior part of holdfast organ, but not into the cup-like walls of the forebody itself, present throughout hindbody, being especially concentrated in its anterior portion. Eggs ellipsoidal, 0.047 to 0.059 × 0.071 (0.073 × 0.048), and present in all specimens. Genital atrium located very near posterior end of body, opening dorsally. Small bursa copulatrix within atrium and extending outward and anteriorly. Male genital pore located in end of bursa copulatrix. Vas deferens sphincter (*"Ringnapf"*) absent. Female genital pore anterior and ventral to bursa copulatrix.

Host: Erolia melanotos, the Pectoral Sandpiper.
Location: Small intestine.
Locality: Near Kemah, in Galveston County, Texas.
Type specimen: U. S. Nat. Mus. Helm. Coll. No. 48789.

DISCUSSION

According to the key and general descriptions given by Dubois (1938), *Strigea intermedia* and *S. nicolli* resemble *S. eroliae* more than do other species of *Strigea.* However, *S. eroliae* differs from *S. intermedia* in the position of the holdfast organ and disposition of the vitellaria. In *S. eroliae,* the holdfast organ protrudes anteriorly and the vitelline follicles of the anterior region are confined to that organ, whereas in *S. intermedia,* the holdfast organ protrudes ventrally and vitelline follicles are found in the cupped walls of the anterior segment as well as in the holdfast organ.

S. nicolli differs from the present species in that the genital atrium is very large and is almost filled by the bursa copulatrix. *S. eroliae* has a small genital atrium and a very small bursa copulatrix.

Harwood (1931) described the North American variety *Strigea falconis meleagris* which resembles *Strigea eroliae* to some extent. However, *S. falconis* has a small holdfast organ, whereas *S. eroliae* has a much larger holdfast. Also, the genital atrium differs in its opening: that of *S. falconis* opens posteriorly and that of *S. eroliae* dorsally.

The present species is the first in the genus *Strigea* to be described from North American shore birds. For that matter, the genus *Strigea* has previously been recorded from shore birds of the suborder *Charadrii* only in Australia.

SUMMARY

Strigea eroliae, n. sp., is described from *Erolia melanotos* and means of distinguishing the species from three rather similar forms are given.

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NOTES ON THE GENUS *MEGARTHROGLOSSUS* (SIPHONAPTERA, HYSTRICHOPSYLLIDAE) IN SANTA FE COUNTY, NEW MEXICO[1]

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Incidental to a field ecology study of rodents and their ectoparasites by the Santa Fe Field Station, 32 specimens of *Megarthroglossus* have been collected from a variety of sources. The following observations are recorded to provide previously unpublished information on the comparative morphology of *Megarthroglossus bisetis* Jordan and Rothschild and *Megarthroglossus divisus divisus* (Baker) together with notes on the host relationships of these little known fleas.

Fleas of the genus *Megarthroglossus* are rarely collected. Hundreds of *Neotoma* and *Peromyscus* have been examined for ectoparasites by the Santa Fe Field Station, yet *Megarthroglossus* have been found only two times on *Neotoma* and one time each on three different species of *Peromyscus*. The few specimens of *Thomomys* and *Ochotona* examined have each provided one host record. Several specimens of *Tamiasciurus* have been examined without finding fleas of the above genus. *Megarthroglossus* have been taken from one of four nests of *Neotoma micropus canescens* and from six of 28 nests of *N. a. albigula*.

MEGARTHROGLOSSUS BISETIS JORDAN AND ROTHSCHILD

Megarthroglossus bisetis Jordan and Rothschild, 1915, Ectoparasites **1**: 54.
Megarthroglossus divisus bisetis Jordan and Rothschild. Hubbard, 1947, Fleas of Western North America, 833 pp., Iowa State College Press.
Megarthroglossus bisetis Jordan and Rothschild. Williams and Hoff 1951, U.S. Nat. Mus. Proc. **101**: 310–311. Description of the male.

The species was described from 2 females collected in August 1900 from *Neotoma* sp. at Beulah (San Miguel County), New Mexico as follows:

"Like *M. longispinus* [=*Megarthroglossus divisus divisus* (Baker)], but with only two antepygidial bristles on each side, the upper bristle being a little (one-fifth or one-sixth) longer than the lower one. The pronotal comb contains sixteen spines. The receptaculum seminis, which unfortunately is collapsed in both our specimens, is similar to that of *M. longispinus,* but the head is more regularly rounded, and the tail apparently somewhat shorter."

Collections from Santa Fe County, New Mexico contain 13 females that agree with the above description. In these specimens the number of pronotal spines was more often 15 but varied from 14 to 18. The posterior margin of sternum VII was irregular and usually without a sinus.

In describing the male of *M. bisetis,* Williams and Hoff failed to mention sternum VIII but stated that the pronotal comb is usually composed of 16 teeth; with a single antepygidial bristle on each side; and sternite IX broad at base, narrowed at apex. Their New Mexico records include one male from *Peromyscus maniculatus* collected near Bernalillo (Sandoval County) and many specimens from *N. m. canescens* or its nests collected near Albuquerque (Bernalillo County).

The 12 males collected in Santa Fe County agree in general with the description of Williams and Hoff. However, all of these specimens have three antepygidial

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[1] From the Communicable Disease Center, Public Health Service, U.S. Department of Health, Education, and Welfare, Atlanta, Ga.

setae on each side. In each case a long conspicuous seta is situated between two relatively minute setae. The number of pronotal spines was 14 on six specimens, 15 on two specimens, and 16 on four specimens. The posterior margin of sternum VIII is convex and the distal arm of sternum IX tapers to a narrowly rounded apex.

Megarthroglossus Divisus Divisus (Baker)

Pulex longispinus Baker 1895, Can. Ent. **27**: 131 132. Described from Georgetown, Colorado, *ex* "Fremont's chickaree" (*Tamiasciurus fremonti*).

Pulex divisus Baker 1898, Journ. N.Y. Ent. Soc. **6**: 54. New name for *P. longispinus* Baker 1895, which was preoccupied by *P. longispinus* Wagner, 1893.

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Megarthroglossus longispinus (Baker). Jordan and Rothschild 1915, Ectoparasites **1**: 52–53. An illustrated redescription.

Megarthroglossus divisus divisus (Baker). Hubbard 1947, Fleas of Western North America, 833 pp., Iowa State College Press. Lists records from a variety of hosts from California, Montana, Utah, and Alberta and states that the Rocky Mountain pine squirrels seem to be the favored host.

Megarthroglossus divisus divisus (Baker). Holland 1949, Canada Department of Agriculture Tech. Bul. **70**: 99. Lists records from British Columbia *ex Neotoma cinerea drummondi* and *Microtus longicaudus mordax*.

Santa Fe County, New Mexico records include five males and two females which are readily identified by the description and illustrations of either Jordan and Rothschild or Holland. Pronotal spines of the two females number 13 and 14; of the males, two have 12 spines, two have 13, and one has 14 spines. The males have one long antepygidial seta between two relatively minute setae.

SANTA FE COUNTY, NEW MEXICO RECORDS

M. d. divisus: 1 male and 1 female *ex Neotoma albigula albigula,* Oct. 26, 1951; 1 male and 1 female *ex* nest of *N. a. albigula,* Jan. 17, 1952; 1 male *ex* nest of *N. micropus canescens,* Mar. 25, 1952; 1 male *ex Peromyscus maniculatus rufinus,* May 6, 1953; and 1 male *ex Ochotona princeps incana,* Aug. 13, 1953.

M. bisetis: 1 female *ex Thomomys talpoides fossor,* Aug. 30, 1951; 1 male *ex Neotoma* sp., Sep. 6, 1951; 1 female *ex* nest of *N. a. albigula,* Oct. 10, 1951; 2 females *ex* nest of *N. a. albigula,* Nov. 29, 1951; 1 male *ex Peromyscus truei truei,* Jan. 11, 1952; 2 males and 2 females *ex* nest of *N. a. albigula,* Jan. 17, 1952; 1 female *ex* nest of *N. m. canescens,* Mar. 25, 1952; 1 male *ex* nest of *N. a. albigula,* Oct. 14, 1952; 5 males and 5 females *ex* nest of *N. a. albigula,* Nov. 18, 1952; 1 male and 1 female *ex* nest of *N. a. albigula,* Jan. 13, 1953; and 1 male *ex Peromyscus nasutus nasutus,* Jan. 22, 1953.

Most of the specimens of *Megarthroglossus* were collected from *Neotoma* nests taken at elevations between 6,300 and 6,900 feet above sea level. Rodents have been trapped at elevations of 5,500 to 11,000 feet. *M. bisetis* was collected at elevations from 5,500 to 9,000; *M. d. divisus* from 6,300 to 11,000 feet.

The few Santa Fe County records of *M. d. divisus* do not indicate any seasonal trend in abundance. Records of *M. bisetis* tend to show a winter peak of abundance and no collections have been made outside of the period from August 30 to March 25. It should be noted that on two occasions both species of fleas were found in a single *Neotoma* nest.

Males of *M. bisetis* and *M. d. divisus* are readily distinguished by the shape of sternum VIII and sternum IX; females by the number of antepygidial setae and

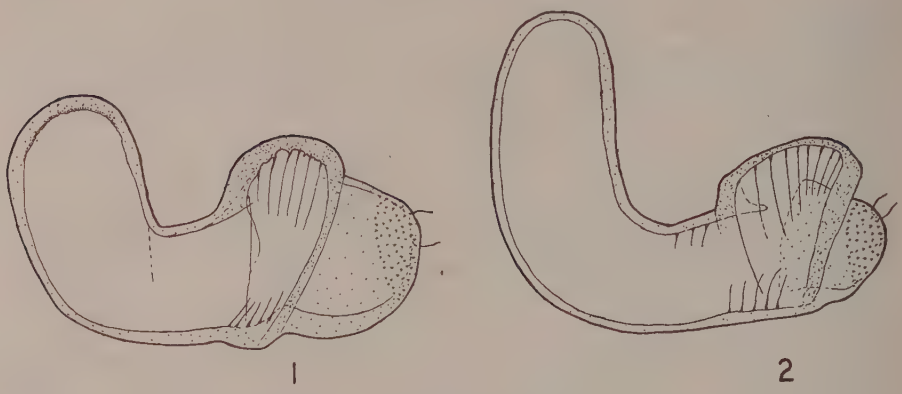

FIG. 1. Spermatheca of *Megarthroglossus bisetis.*
FIG. 2. Spermatheca of *Megarthroglossus divisus divisus.*

the shape of the spermatheca (receptaculum seminis). The posterior margin of sternum VIII of *M. bisetis* is convex; in *M. d. divisus* it is sinuate with a subventral lobe. In *M. bisetis* the distal arm of sternum IX tapers to a narrowly rounded apex; in *M. d. divisus* it is broadly rounded at the apex. There are two antepygidial setae on each side in females of *M. bisetis;* three in females of *M. d. divisus.* The spermatheca in *M. bisetis* (Fig. 1) has a relatively large head, narrow "collar," and short tail which may be slightly expanded subapically; in *M. d. divisus* (Fig. 2) the head is smaller, the "collar" broader, and there is no subapical expansion of the relatively longer tail.

GIGANTORHYNCHUS ORTIZI N. SP., AN ACANTHOCEPHALAN FROM *METACHIRUS NUDICAUDATUS*[1]

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In 1951, while on an expedition into the jungle in La Merced, Peru, Javier Ortiz de la Puente, ornithologist at the Museum of the University of San Marcos, Lima, Peru, collected a specimen of the rat-tailed marsupial, *Metachirus nudicaudatus.* The intestine of this animal contained 24 acanthocephalans which proved on further examination to belong to the genus *Gigantorhynchus.* Only 1 species, *G. echinodiscus* Diesing, was known in that genus until Machado (1941) described a second species, *G. lutzi,* from a Brazilian marsupial, *Caluromys philander.* The acanthocephalan here recorded is the first to be reported from *M. nudicaudatus* and is described as *Gigantorhynchus ortizi* n. sp. The specific name is in honor of the late Doctor Javier Ortiz.

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[1] A part of a thesis submitted to the graduate faculty of the Virginia Polytechnic Institute in partial fulfillment of the requirements for the Master's degree.

The writer wishes to acknowledge her indebtedness to Dr. W. L. Threlkeld for his encouragement, kindness, and helpful suggestions, and under whose supervision this work was carried out.

Dr. Machado very kindly sent a male specimen of *Gigantorhynchus lutzi* to the author for examination. Because of its condition, however, the specimen could not be readily compared with the parasite here reported.

MATERIALS AND METHODS

All specimens were fixed 8 days in 10 percent formalin, and then preserved in 70 percent alcohol. Different stains were used in order to show the complete morphology. The stains employed were picrocarmine, Mayer's carmalum, Reynold's stain, and Delafield's hematoxylin slightly acidified with acetic acid. Specimens were cleared in either xylene or wintergreen and mounted in permount. Two worms were prepared as serial sections stained with Delafield's hematoxylin-eosin.

The hooks were measured by means of a camera lucida. The following method was employed: the length of the hooks with double root from the base "C" to the tip following the median curvature of the hook, and the length of the root from the distal end "A" to the proximal end "B"; likewise, the hooks with simple root from the origin "D" to the tip including the root.

Gigantorhynchus ortizi n. sp.
(Figures 1–10)

Specific diagnosis: With the characters of the genus *Gigantorhynchus* Hamann, 1892. Body large and cylindrical, with imbricated pseudosegmentation, and divided into 3 portions by a dorsal and 2 lateral longitudinal grooves. A collar-like structure is present at 1.80 mm. posterior to the cuticular ring or neck fold. Body of females attenuated in both extremities measure 130 to 242 mm. in length and 1.50 to 2 mm. in maximum dorsoventral diameter. Males curved posteriorly measure 46 to 75 mm. long and from 1.4 to 1.92 mm. in maximum diameter at approximately the level of the cement glands.

The proboscis in both sexes is cylindrical and curved ventrally, neck short and devoid of hooks. Retractile praesoma is 1.45 to 1.72 mm. long and 0.435 to 0.555 mm. wide.

The proboscis is armed at the summit with 2 rows of 6 hooks each with double roots (Fig. 1). The dimensions of these hooks from their base "C" to the tip is approximately 0.160 mm. for the first row and 0.140 mm. for the second row. The length of the roots "A" to "B" is 0.100 mm. in the first row and 0.090 mm. in the second row (Fig. 3). The posterior portion of the proboscis is armed with small hooks with a simple root (Fig. 6), averaging about 0.050 mm. long. There are eleven or twelve of these hooks in each longitudinal row. According to the method of Van Cleave[2] (1941), the number of circles of hooks in this specimen is 21 or 23. The proximal circlets consist of numerous small hooks which increase in length as they approach the equatorial zone and decrease in length and number at the distal portion (Fig. 4).

[2] "In the writings of nineteenth century workers there is still confusion in the interpretation of their descriptions, whether they were recording the number of alternating circles of hooks or the number of hooks in each longitudinal row. The number of circles is twice the number of hooks in one longitudinal row, plus or minus one, for often adjacent vertical rows differ by one hook. Among present day morphologists it is very generally the practice to express the formula of proboscis hooks in number of longitudinal rows and number of hooks in each row, indicating the observed range of variability in both counts."

The proboscis receptacle is a closed muscular pouch suspended inside the anterior end of the proboscis. This pouch measures from 0.750 to 0.920 mm. long and does not receive the proboscis when it is inverted. In the specimen here described, the receptacle extends caudally half the length of the praesoma. Within the cavity are the invertor and retractor muscles, and between the invertor muscles lies a pyramidal mass of nerve cells which constitute the brain (Fig. 5).

The entire structure of the specimen, from the anterior tip to the cuticular ring, is here described as the praesoma, which includes the true proboscis and the neck (Van Cleave, 1950).

The lemnisci are long and cylindrical; they measure from 5.48 to 6.80 mm. in length by 0.129 to 0.163 mm. in width. The number of nuclei per lemniscus varies from 5 to 6. In the same specimen (Fig. 7) the number of nuclei observed in one lemniscus was 5, and 6 in the other.

A special arrangement of subcuticular nuclei of a fragmented type form the collar-like structure (Fig. 7) of the anterior trunk region. In addition to these nuclei, several other types of subcuticular nuclei have been observed throughout the body of the parasite. Immature specimens show spherical and ameboid types (Fig. 2), and older specimens display fragmented nuclei resembling those in the collar.

Males: The genital organs of the males are located in the posterior fifth of the body. The testes are elliptical and measure from 1.98 to 3.00 mm. long by 0.56 to 0.920 mm. wide. Just posterior to the testes are the 8 cement glands, closely grouped, but possessing distinctive walls and a single nucleus each (Fig. 10). The 2 vas efferentia unite within the cement glands to form a common sperm duct. The seminal vesicle and ducts of the cement glands are enclosed in the genital sheath. The penis projects into the bursa which is eversible.

Females: The ovary is represented by numerous small ovoid fragments, 0.150 to 0.270 mm. wide. The genital complex conforms with that usually described for this family. The vagina is short and muscular (Fig. 8). The eggs are elliptical and surrounded by 4 membranes, the exterior of which is smooth, but the shell within it is wrinkled. The eggs measure from 0.079 to 0.085 mm. in length by 0.049 to 0.054 mm. in width (Fig. 9).

Host: Metachirus nudicaudatus

Site: Intestine

Locality: Peru, South America

Type Specimen: U. S. Nat. Mus. Coll. No. 37433.

DISCUSSION

Of the two described species of *Gigantorhynchus, G. lutzi* is the only one which *G. ortizi* resembles enough to warrant further discussion.

The over-all length of *G. ortizi* exceeds that reported by Machado (1941) for *G. lutzi,* and the species differ in external appearance. *G. lutzi* is described as having 2 longitudinal grooves, a dorsal and a ventral, whereas in *G. ortizi* there are 1 dorsal and 2 lateral grooves. A further distinguishing feature of *G. ortizi* is the presence of a nuclear collar at the anterior portion of the trunk; such a collar is not mentioned for the other species of *Gigantorhynchus.*

The hooks at the summit of the proboscis in *G. ortizi* are smaller than in *G. lutzi,* whereas the hooks with simple roots are larger on the average. The arrangement

of small proboscis hooks also differs from that shown in Machado's drawing. In *G. ortizi*, the hooks are numerous and smaller in the first 6 or 7 circlets, and decrease in number at the equatorial zone. The number of these hooks in *G. lutzi* was not stated by Machado.

In Machado's figure of *G. lutzi*, the proboscis receptacle extends posteriorly as far as the neck fold, while in *G. ortizi* this pouch is much shorter and displays a cleft in the ventral side. In *G. lutzi*, the brain is at the posterior end of the pouch whereas in *G. ortizi*, the brain is not terminal. The lemnisci of these species differ both in relation to the length of the body and in respect to the number of their nuclei.

Marked differences in the genital organs of these species have also been found. In *G. ortizi* the testes are smaller, the cement glands are compact and not separated as in *G. lutzi*, and the ovarian fragments are smaller.

SUMMARY

A new species of Acanthocephala, *Gigantorhynchus ortizi* from *Metachirus nudicaudatus*, has been described and comparisons of its morphology have been made with the species of *Gigantorhynchus* (*G. echniodiscus* and *G. lutzi*).

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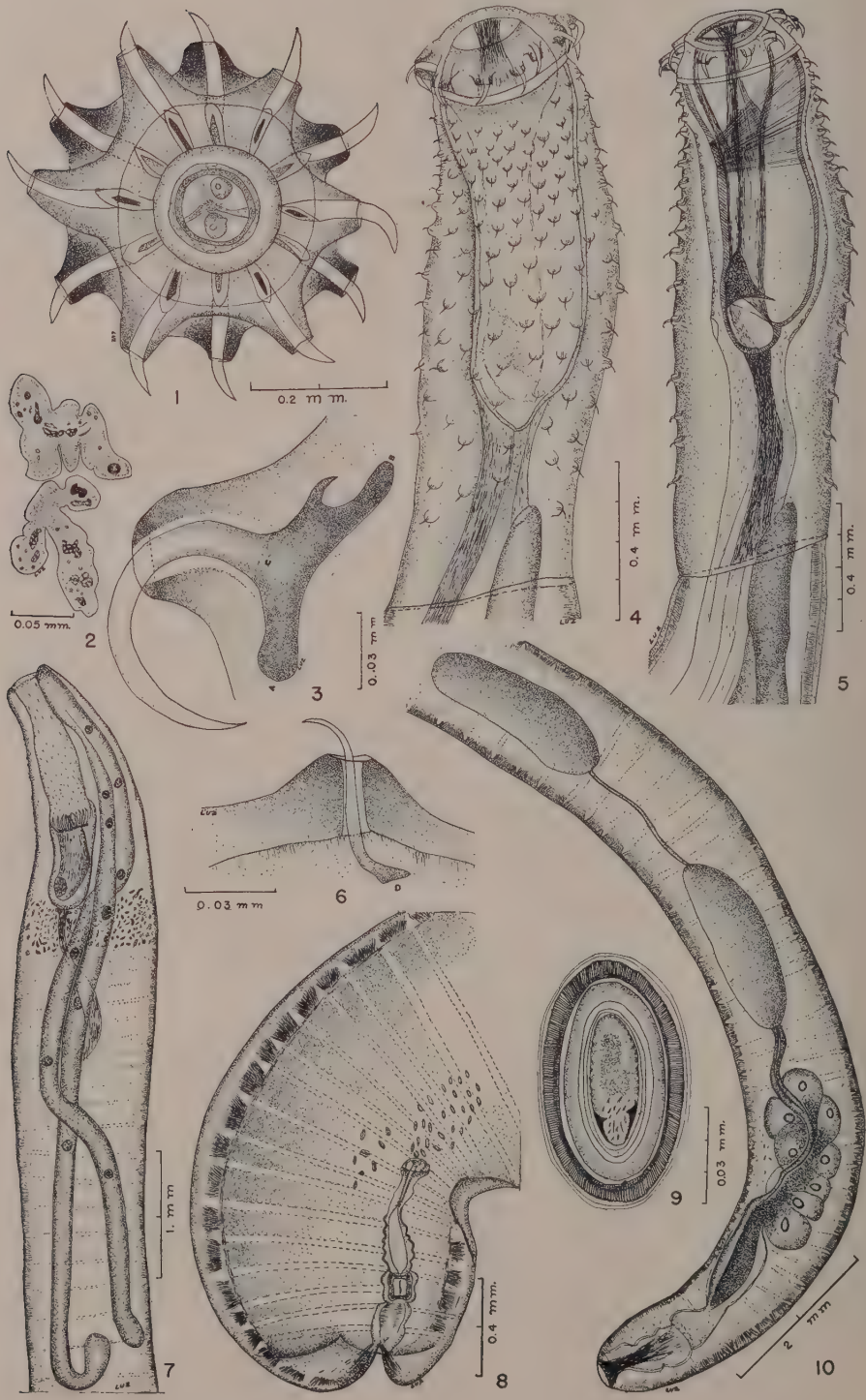
EXPLANATION OF PLATE

All figures concern *G. ortizi* n. sp. Figure 4 was made with the aid of a projector and other figures with a camera lucida.

FIG. 1. Apical view of the proboscis showing the hooks with double root.
FIG. 2. Subcuticular ameboid nuclei.
FIG. 3. Hook with double root.
FIG. 4. Proboscis showing the arrangement of hooks.
FIG. 5. Praesoma showing proboscis receptacle and associated structures in detail.
FIG. 6. Hook with simple root.
FIG. 7. Anterior portion showing inverted proboscis, lemnisci, and collar-like structure of nuclei.
FIG. 8. Posterior end of female.
FIG. 9. Egg showing smooth external membrane and wrinkled shell.
FIG. 10. Posterior end of male showing arrangement of genital organs.

PLATE I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10

SUSCEPTIBILITY OF SNAIL VECTORS TO GEOGRAPHIC STRAINS OF *SCHISTOSOMA JAPONICUM*[1]

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In recent years several investigators have reported finding differences in the susceptibility of snail vectors to the schistosomes of man. Vogel (1941) reported that *Planorbis pfeifferi* (*Biomphalaria pfeifferi*) from West Africa was more susceptible to *Schistosoma mansoni* of West African origin than *Planorbis guadaloupensis* (*Australorbis glabratus*) from Dutch Guiana. Stunkard (1946) and Cram, Files, and Jones (1947) reported finding that *Planorbis boissyi* (*Biomphalaria boissyi*) from Egypt was refractory to *S. mansoni* from the West Indies. In addition, Files and Cram (1949) found that while *Australorbis glabratus* from Puerto Rico served adequately for the Puerto Rican strain of *S. mansoni*, *A. glabratus* from São Salvador, Brazil was completely refractory. Newton (1953) demonstrated that susceptibility to *S. mansoni* in *A. glabratus* is a heritable character.

In experiments with *S. haematobium* Cowper (1947) found that *Bulinus truncatus* of Egyptian origin had no attraction for miracidia of a strain from South Africa, and Barlow and Abdel-Azim (1948) reported that *Physopsis africana* from South Africa was susceptible to the Egyptian strain, whereas, *Bulinus tropicus* from South Africa was refractory.

The findings cited above, in addition to others reported, indicate that there are geographic strains of *S. mansoni* and *S. haematobium*, as well as certain snail hosts which, while morphologically identical with other strains, possess physiological differences affecting the host–parasite relationship.

The present paper reports a series of experiments, designed to yield information concerning the susceptibility of several snail hosts to *S. japonicum* from 3 distinct geographical areas.

While this work was in progress, Hunter, Ritchie and Otori (1952), working independently, found that wild specimens of *Oncomelania formosana* (Formosa) were unsuitable as hosts for *S. japonicum* autochthonous to Japan.

MATERIAL AND METHODS

Laboratory-reared specimens of 5 species of snails were employed in these experiments. The laboratory colonies were established from adult snails obtained as follows: *Oncomelania hupensis* from Shanghai, China, *O. nosophora* from Kofu, Japan, *O. formosana* from Chang Hua, Formosa, *O. quadrasi* from the islands of Leyte and Mindanao, in the Philippines, and *Pomatiopsis lapidaria* from along the banks of the Potomac River near Washington, D.C. *P. lapidaria* was included in this study because of its having been previously experimentally infected (Stunkard, 1946; Berry and Rue, 1948; and DeWitt, 1952) with *Schistosoma japonicum*.

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[2] With the technical assistance of Rodney H. Duvall.

[3] Laboratory of Tropical Diseases.

Schistosoma japonicum used in these experiments was obtained from infected snails included in the snail shipments from China, Japan, and Formosa, as listed above. The life cycle of each strain was established and maintained in the laboratory with the use of small mammals and snails from the laboratory breeding colonies.

Snail exposures were made with miracidia hatched from ova which were freed from infected liver tissue. Only freshly hatched miracidia were used. Snails were exposed either individually to 10 miracidia or in pairs to 15 miracidia. The exposures were made in small Syracuse dishes, allowing 12 to 14 hours for penetration. Tests were made in several series, using various numbers of snails; therefore the total number of snails exposed to any specific strain represents an aggregate of several experiments. An attempt was made to include a cross section of snails ranging in size from juveniles to fully matured specimens.

After exposure, the snails were kept in clay saucers or glass bowls which contained moistened dirt, leaves and twigs. From time to time small amounts of tropical fish food were added. Sixty days after exposure the snails were isolated in small tubes of water for observation. Those snails found to be negative for cercaria were returned to the saucers and held for 5 subsequent examinations at weekly intervals. At the end of this observation period the snails still remaining negative were examined by dissection; those found to contain cercariae were considered as positive.

RESULTS

A total of 303 *O. hupensis,* 313 *O. nosophora,* 499 *O. quadrasi,* 644 *O. formosana,* and 1208 *P. lapidaria* were exposed to *S. japonicum* from China, Japan, and Formosa. The results of the exposures, which are shown in Table 1, reveal that

TABLE 1.—*Results following exposure of* Oncomelania hupensis, O. nosophora, O. formosana, O. quadrasi, *and* Pomatiopsis lapidaria *to* Schistosoma japonicum *from China, Japan, and Formosa*

Source of *S. japonicum*	*Oncomelania hupensis* from China				*Oncomelania nosophora* from Japan				*Oncomelania quadrasi* from Philippines				*Oncomelania formosana* from Formosa				*Pomatiopsis lapidaria* from U.S.A.			
	E	L	+	%	E	L	+	%	E	L	+	%	E	L	+	%	E	L	+	%
Shanghai, China	64	53	18	34	85	68	0	0	114	98	0	0	154	150	0	0	480	345	4	1
Kofu, Japan	131	113	15	13	105	87	18	21	105	91	0	0	136	123	0	0	564	499	0	0
Chang Hua, Formosa	108	97	0	0	123	67	14	21	280	245	1	0.4	354	315	111	35	164	107	3	3

E = Total number of snails exposed.
L = Total number of snails alive at end of observation period; 90–100 days.
+ = Number of snails positive for *S. japonicum.*
% = Percentage of snails positive as based on the total number of snails alive at the end of the observation period.

O. hupensis (China) became infected with *S. japonicum* from China and Japan, but was refractory to *S. japonicum* from Formosa. *O. nosophora* (Japan) became infected with Japanese and Formosan strains but could not be infected with the Chinese strain. *O. quadrasi* (Philippines) was completely resistant to the development of the Chinese and Japanese strain, and only a very small percentage of the snails exposed to the Formosan strain became infected. *O. formosana* (Formosa) was readily infected with its native Formosan strain, but was refractory to the other two strains. Small numbers of *Pomatiopsis lapidaria* became infected with both Chinese and Formosan strains, but no evidence of infection was found when they were exposed to the Japanese strain.

DISCUSSION

The experiments reported in this paper contribute information on the compatibility of parasite and snail host in the life cycle of *Schistosoma japonicum*. Snail vectors from some endemic areas were found to be capable of serving as the intermediate host of *S. japonicum* from more than one geographical area, while others were not so broadly adapted. This is diagrammatically shown in figure 1. In addition evidence was obtained that suggests possible basic physiologic differences between *S. japonicum* of different endemic areas. For example, it was found that *S. japonicum* from Shanghai, China could infect *O. hupensis* from the same area, but not *O. nosophora* from Kofu, Japan, while *S. japonicum* from Kofu, Japan could infect snails from either area, and *S. japonicum* from Chang Hua, Formosa could infect *O. nosophora* from Kofu, Japan but not *O. hupensis* from Shanghai,

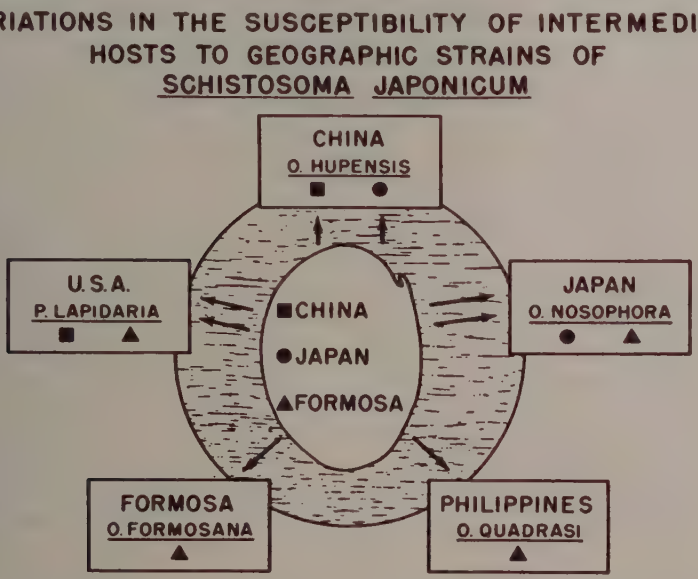

FIG. 1. Variations in the susceptibility of intermediate hosts to geographic strains of *Schistosoma japonicum*.

China. From this, it seems reasonable to conclude that the three strains of parasites are physiologically different.

It can be noted from the table that 5 species of snails were exposed to 3 strains of *S. japonicum*, resulting in 15 different snail-parasite combinations. Of the 15 groups, 8 were found to have some degree of compatibility. Among these 8 groups, 3 were found to have infection rates of 3 per cent or less while the others ranged from 13 to 35 per cent. This would seem to suggest that compatibility between snail and parasite is one of degree, and that the degree of compatibility is dependent on physiologic characteristics of both host and parasite.

SUMMARY

In this study, laboratory-reared *Oncomelania hupensis* originating from Shanghai, China; *O. nosophora* from Kofu, Japan; *O. formosana* from Chang Hua, For-

mosa; *O. quadrasi* from Leyte and Mindanao, P.I.; and *Pomatiopsis lapidaria* from the Potomac River near Washington, D.C. were exposed to strains of *Schistosoma japonicum* from Shanghai, China; Kofu, Japan; and Chang Hua, Formosa. *O. hupensis* was readily infected with the Chinese and Japanese strains but in these tests could not be infected with the Formosan strain. *O. nosophora* was susceptible to both the Japanese and Formosan strains but was refractory to the Chinese strain. *O. formosana* became infected with the Formosan strain but could not be infected with the Chinese and Japanese strains. *O. quadrasi* was susceptible only to the Formosan strain while *P. lapidaria* was infected with the Chinese and Formosan strains but not the Japanese strain.

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EXPERIMENTAL INFECTION OF THE AFRICAN RELAPSING FEVER TICK, *ORNITHODOROS MOUBATA* (MURRAY), WITH *BORRELIA LATYCHEVI* (SOFIEV)

Willy Burgdorfer[1] and Gordon E. Davis[2]

The Asiatic relapsing fever spirochete, *Borrelia latychevi*, was isolated by Sofiev (1941) in Middle Asia from rodents, *Rhombomys opimus* and *Gerbillus eversmanni*, and from its natural vector, the argasid tick, *Ornithodoros tartakovskyi*. Recently, Baltazard and collaborators (1952) reported the unsuccessful attempts to transmit this spirochete by *O. erraticus* (small and large form) and *O. normandi*

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and stated that *O. tholozani* and *O. moubata*, as well as the North American argasid ticks, could not serve as vectors of this organism.

In studies of the life cycle of spirochetes in various tick vectors, the development and persistence of *B. latychevi* in *O. moubata* were observed. It is the purpose of this paper to report these observations which are partially in disagreement with those of Baltazard *et al.*

MATERIALS AND METHODS

The *O. moubata* used for the following experiments were from clean stock maintained at the Rocky Mountain Laboratory. This stock originated from ticks collected in the Belgian Congo by Dr. R. Geigy, Director of the Swiss Tropical Institute. Dr. M. Baltazard, Director of the Pasteur Institute, Teheran, Iran, kindly sent us infected *O. tartakovskyi* from which a strain of *B. latychevi* was isolated. This species of relapsing fever spirochete produces only a mild infection in adult rabbits, white mice, and man and fails to infect white rats, gunea pigs, or dogs (Sofiev, 1941; Pospelowa-Shtrom, 1940). New-born rabbits, however, are highly susceptible, since from 70 to 100 per cent of these animals, infected either by tick bite or by inoculation of infectious blood, die in 15 to 20 days. During this period, a steady increase in the number of spirochetes in the peripheral blood can be observed (Baltazard *et al.*, 1952).

In order to carry out the experiments to be described, large numbers of ticks and infected hosts were needed. The following procedure was employed. Infected *O. tartakovskyi* were allowed to feed on a normal white mouse. Tail blood of this animal was examined daily. As soon as spirochetes were observed, the blood was used to inoculate groups of normal mice. When these animals were known to have spirochetemia, second and fourth nymphal stages and adults of *O. moubata* were allowed to feed on them. After the ticks had engorged, they were stored at room temperature in desiccator jars in which a relative humidity of 79 per cent was maintained by a saturated solution of ammonium chloride. In order to follow the behavior of *B. latychevi* in *O. moubata*, several ticks were dissected each day and their organs were examined by dark-field technique according to methods described by Burgdorfer (1951).

In addition to this procedure, haemolymph and coxal fluid from the ticks were examined for spirochetes. The haemolymph was obtained by amputating the distal portion of the legs, the coxal fluid by applying a warm needle or forceps to the ventral side of the ticks, which respond to such irritation with the expulsion of this fluid.

Smears prepared from the blood of infected mice were examined under the microscope, using a magnification of 450 and ordinary direct illumination.

RESULTS

1. *Infection and persistence of* B. latychevi *in second nymphal stage* O. moubata. Two hundred *O. moubata* in the second nymphal stage were fed on a mouse which had in the peripheral blood an average of two to three spirochetes per microscopic field. Fifty-three of 90 ticks examined during the first 75 days after the infective feeding were shown to be carriers of active spirochetes. The organisms were regularly found in the haemolymph, central ganglion, coxal organs, and salivary glands after the fifth day following the infective feeding. An increasingly heavy infiltra-

tion could be observed in the central ganglion and in the coxal organs, whereas in the salivary glands fewer spirochetes were found as the period of infection increased.

2. *Infection and persistence of* B. latychevi *in fourth nymphal stage* O. moubata. One hundred and ten *O. moubata* in the fourth nymphal stage were fed upon mice which had in the peripheral blood an average of four spirochetes per microscopic field. Twenty days after the ticks had engorged, the survival of *B. latychevi* was checked by examination of haemolymph. Spirochetes in varying numbers were found in 70 of 110 ticks studied. All of the ticks which did not show spirochetes in the haemolymph were dissected and examined. Heavy infections were noted in central ganglions, coxal organs, and salivary glands of 15 of these 40 ticks. Spirochetes could also be found in the walls but never in the lumen of the Malpighian tubules. Direct examinations of ticks previously shown to be infected revealed that *B. latychevi* persists in the tissues for more than 150 days.

3. *Infection and persistence of* B. latychevi *in adult* O. moubata. Fifty adult *O. moubata,* both males and females, were fed on infected mice whose peripheral blood contained an average of three spirochetes per microscopic field. Thirteen of the ticks were dissected and tested at intervals from 10 to 340 days after the infective

TABLE 1.—*Transovarial transmission. Presence of* B. latychevi *in eggs and progeny of infected* O. moubata *as revealed by dark-field examination*

Tick No.	Date of Infection	Date of Oviposition	No. of Eggs			No. of Nymphs		
			Tested	Positive	Negative	Tested	Positive	Negative
1	7–7–52	8–13–52	33	16	17	None		
2	"	9–15–52	50	0	50	25	0	25
3	12–6–52	4–5–53	30	30	0	20	12	8
4	"	"	15	12	3	20	12	8
5	"	4–6–53	15	15	0	25	17	8
6	"	"	15	14	1	15	1	14
7	"	6–14–53	21	15	6	None		
8	"	"	26	18	8	20	15	5

feeding. It was found that *B. latychevi* developed and persisted also in adult *O. moubata.* The spirochetes penetrate the gut wall, enter the body fluid, and then attack the various organs. Central ganglion, coxal organs, salivary glands, and the walls of the Malpighian tubules are regularly parasitized. In addition, the spirochetes were found in the tissues of the genital system of adult female ticks, especially in the ovarian tissue and egg follicles. The genital system of male ticks was not examined. Examination of eggs from 8 and of nymphs from 6 infected female ticks indicated that transovarial transmission can occur. Spirochetes could be found in eggs of 7 and in nymphs of 5 infected *O. moubata* (see Table 1).

4. *Transmission experiments.* Theoretically, *O. moubata* could transmit relapsing fever spirochetes by (a) excretions of the Malpighian tubules, (b) coxal fluid, which is ejected shortly before the tick finishes feeding, and (c) saliva, *i.e.,* by bite. Although the spirochetes were found in each of the organs which secrete these fluids, transmission through the excretions of the Malpighian tubules would not be expected since no spirochetes were found in the lumen of this organ or in the so-called "tick feces."

The coxal fluids of several hundred *O. moubata* were examined for the presence of spirochetes. With a few exceptions all fluids contained spirochetes in varying numbers. Seventy-three transmission experiments were then performed to determine whether or not infected *O. moubata* could transmit *B. latychevi* to adult white

TABLE 2.—*Number of spirochetes in peripheral blood of adult mice, suckling mice, and new-born rabbits inoculated with* B. latychevi

	Number of spirochetes* found																	
	Days after inoculation																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Adult mice																		
No. 1	0	0	1	45	3	4	1	4	14	8	1	–	1	1	0	0	1	0
No. 2	0	0	1	59	4	1	1	3	4	0	0	–	0	0	0	0	0	0
Suckling mice																		
No. 1	0	1	1	15	92	7	43	52	47	4	48	–	15	11	5	14	4	8
No. 2	0	1	2	27	79	32	39	39	140	4	12	–	14	12	0	1	1	8
New-born rabbits																		
No. 1	0	0	20	202	300	300	104	130	21	108	164	+						
No. 2	0	1	16	180	140	300	300	80	120	+								

– = Not examined.
+ = Died.
* The number of spirochetes was estimated by counting the organisms observed over a period of 5 minutes in a blood smear stained by Giemsa's method.

mice or new-born rabbits. Ticks in lots of 3 to 10 were employed in each experiment. None of the animals became infected.

To explain the negative results, it was postulated that either the quantity of spirochetes transmitted by saliva and coxal fluid was not sufficient to produce a frank infection in the animals or the virulence of *B. latychevi* had been decreased by passage through *O. moubata*. In order to determine which of the above explanations was valid, a comparative study was made of the course of infection in experimental animals inoculated either with suspensions of infected *O. moubata* or with blood from a mouse upon which infected *O. tartakovskyi* had fed.

Six suckling mice, 6 adult mice, and 6 new-born rabbits were inoculated with 0.25 ml. of citrated blood (12 drops of tail blood containing one spirochete per microscopic field, diluted in 2.0 ml. citrate solution) from a mouse which had been infected by *O. tartakovskyi*. All animals became infected. The course of infection in the peripheral blood of two animals of each group is shown in Table 2. It was found that the number of spirochetes in the blood of adult mice was less than the number in the blood of suckling mice and that spirochetemia persisted for a greater length of time in the latter animals. In new-born rabbits, however, an extremely heavy infection occurred, which caused death of all the animals.

In a parallel experiment, the animals were inoculated with suspensions of infected *O. moubata*. The ticks had been fed on infected mice and were then held for periods of 90 to 355 days before they were used. Varying numbers of ticks were triturated in 1.0 to 3.0 ml. of saline, and 0.25 ml. of the suspension, which was proved to contain spirochetes, was inoculated intramuscularly into suckling mice,

TABLE 3.—*Results of attempts to infect mice and rabbits by injecting suspensions of* O. moubata *infected with* B. latychevi

No. of triturated ticks	Days after infective feeding	Suckling mice		Adult mice		New-born rabbits	
		Positive	Negative	Positive	Negative	Positive	Negative
10	92	0	6	–	–	–	–
10	92	–	–	1	5	–	–
2	104	–	–	0	6	0	2
1	138	–	–	0	4	–	–
2	184	–	–	–	–	1	1
1	210	–	–	3	4	–	–
4	212	–	–	–	–	0	4
3	212	–	–	–	–	1	3
1	355	–	–	–	–	1	3

– = None injected.

adult mice, and new-born rabbits. As will be noted in Table 3, only a few animals became infected and in most instances the inocula did not produce infection.

DISCUSSION

Since *B. latychevi* persisted and multiplied in the tissues of *O. moubata,* including the coxal organs and salivary glands, there was a possibility that this species of tick would transmit these spirochetes as did *O. tartakovskyi.* However, none of the mice or new-born rabbits used in transmission experiments became infected, and a reason for the apparent discrepancy was sought. *B. latychevi* is relatively less virulent than other species of relapsing fever spirochetes, but it does cause a frank infection in mice and particularly in new-born rabbits when transmitted by its natural vector, *O. tartakovskyi,* or when infected blood is employed as an inoculum (see Table 2). The fact that suspensions of heavily infected *O. moubata* inoculated into these animals failed, with a few exceptions, to produce an infection suggests that *B. latychevi,* by passage through *O. moubata,* partially loses its original virulence.

SUMMARY

B. latychevi persists through all stages of *O. moubata* studied. It is found consistently in the central ganglion, the coxal organs, the salivary glands, the walls of the Malpighian tubules, and in the tissues of the genital system, as well as in the progeny of infected ticks.

Attempts to infect mice and new-born rabbits by feeding infected *O. moubata* were unsuccessful, and inoculation of suspensions of heavily infected ticks into these animals failed, except in a few instances, to produce infection. The results of these experiments indicate, therefore, that there is a change in the virulence of *B. latychevi* induced by passage through *O. moubata.*

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THE EFFECTS OF VARIOUS ENVIRONMENTAL FACTORS ON THE HATCHING OF EGGS OF *PLAGITURA SALAMANDRA* HOLL (TREMATODA: PLAGIORCHIIDAE)[1]

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Plagitura salamandra Holl, 1928 is an intestinal parasite of the spotted newt, *Triturus v. viridescens* (Rafinesque) and uses the snail *Pseudosuccinea columella* (Say) as its intermediate host. *P. salamandra*, like all the digenetic trematodes, has a complex life history, but it deviates from the generalized life cycle primarily by the manner in which the miracidia are liberated.

The eggs of *P. salamandra* containing mature miracidia are ingested by a specific snail host in the digestive tract of which the eggs hatch. The liberated miracidia move through the intestinal wall and the developing larvae make their way to the hepatic gland (Owen, 1946) The life cycle then follows the generalized one. Though this deviation from the generalized cycle (i.e., in which the eggs hatch in the water and the miracidia penetrate the body of the snail) is not peculiar to *Plagitura salamandra*, studies on trematodes have not contributed materially to an understanding of the factor or factors influencing the liberation of miracidia in the intestinal tract.

A series of experiments was undertaken to attempt to hatch eggs of *P. salamandra in vitro*, and to ascertain, if possible, the factors necessary for hatching. These experiments were designed to determine the following:

1. The effect of extracts prepared from the digestive tracts of host specific snails and from non-specific aquatic snails on the hatching of eggs.
2. The effect of NaCl and KCl solutions of varying concentrations ranging in pH from 4.0–8.6 on the hatching of eggs.

MATERIALS AND METHODS

Preparation and examination of eggs.

Gravid trematodes obtained from the intestinal tract of newts were placed in 0.6% NaCl for 1 hour, then changed into tap water following which the eggs were rapidly discharged. Many of the eggs so obtained contained embryos and were kept in tap water for 4 to 5 days until actively motile miracidia were observed within the shell.

Forty to 50 eggs containing actively moving larvae were used in all experiments. The eggs (in 0.01 ml. of water) were placed in depression slides which contained 0.01 ml. of the various solutions to be tested. Solutions were replaced every 24 hours. All series were incubated in a moist chamber at room temperature (18°–21° C.) unless otherwise indicated. Eggs were examined daily for 5 days to determine hatching and motility of the liberated larvae. Hatching was recorded only when 20% or more of the eggs hatched.

[1] Contribution from the Miller School of Biology, University of Virginia. Part II of a dissertation submitted in candidacy for the Ph.D. degree.

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Preparation and testing of extracts of snail digestive tracts.

Aqueous extracts of digestive tracts were made from 25 of each of the following species of snails: *Pseudosuccinea columella* (Say), *Physella heterostropha* (Say), *Helisoma anceps* (Menke), *Oxytrema virginica* (Gmelin) (= *Goniobasis virginica*), *Nitocris carinata* (Brugiere). Digestive tracts were removed and ground with washed sterile sand in 0.5 ml. of triple distilled water. The homogenate, infused at 4° C. for 2–4 hours, was centrifuged and the supernatant fluid removed and used as the "extract." Immediately before using, each extract was divided into two equal portions and further diluted: one with an equal volume of distilled water and the second with an equal volume of 0.6% NaCl.

The eggs were placed in an aqueous extract and in one to which an equal volume of 0.6% NaCl (final concentration of NaCl = 0.3%) had been added. Controls of eggs in distilled water and 0.3% NaCl were maintained simultaneously.

Preparation and testing of the NaCl and KCl solutions.

Solutions of NaCl and KCl ranging in concentration from 0.1% to 1.0% were prepared in Clark and Lubs Buffers. The buffer solutions used for the NaCl dilutions had the following pH range: 4.0, 5.0, 7.0, and 7.6; while the KCl dilutions were made in buffers ranging from pH 4.0, 5.0, 7.0, 8.0, to 8.6. Similar concentrations were prepared with NaCl and KCl in monobasic and dibasic potassium phosphate buffers with a pH range of 5.0, 7.0, and 7.6. A Beckman model G meter was used for all pH determinations.

Eggs were placed in the buffered NaCl and KCl solutions and at the same time eggs were placed in the buffer solutions alone.

RESULTS OF EXPERIMENTS

Effect of extracts of snail digestive tracts.

The eggs maintained in the extract of *P. columella* hatched, as did also those eggs in the extract (0.3% NaCl) of three of the non-specific snails (Table 1). Al-

TABLE 1.—*Effect of digestive tract extracts of various snails, with and without the addition of NaCl on the hatching of eggs containing active miracidia at 18°–20° C.*

Snail Species	Aqueous Extract	NaCl & Extract
P. columella	2* M**	1M
P. heterostropha	–	1
H. anceps	–	1
O. virginica	–	3
N. carinata	–	–
Controls:	Distilled water	0.3% NaCl
	–	–

* Number refers to days of incubation before hatching.
** M indicates that some of the miracidia were motile after leaving egg shell.

though the extract of the non-specific snail plus the NaCl did result in hatching, none of these miracidia exhibited motility. The only motility observed was among some of those miracidia which were liberated in the extract of the host specific snail, *Pseudosuccinea columella.*

Effect of varying concentrations of NaCl in buffered solutions.

Some of the eggs hatched in all the NaCl solutions (Clark and Lubs Buffer) except in those with a pH of 4.0 and the one in which a 0.1% NaCl solution was buffered to a pH of 5.0 (Table 2). The eggs in concentrations of NaCl above 0.6%

TABLE 2.—*Effect of NaCl prepared in Clark and Lubs buffers and potassium phosphate buffers on the hatching of eggs containing active miracidia at 18°–21° C.*

NaCl Conc.	*Clark and Lubs Buffers*				*Potassium Phosphate Buffers*		
	pH 4.0	pH 5.0	pH 7.0	pH 7.6	pH 5.0	pH 7.0	pH 7.6
0.1%	–	–	3*M**	2 M	–	–	–
0.2%	–	3 M	3 M	2	–	–	–
0.3%	–	3 M	3 M	3	–	–	–
0.4%	–	3 M	3 M	3	–	–	–
0.5%	–	3	2 M	3	–	–	–
0.6%	–	3	2 M	3	–	–	–
0.7%	–	3	2	3	–	–	–
0.8%	–	3	2	3	–	–	–
0.9%	–	3	2	3	–	–	–
1.0%	–	3	2	3	–	–	–
Controls:							
Buffer only	–	–	2	–	–	2	–

* Number refers to days required for eggs to hatch.
** M indicates that some of the miracidia were motile after hatching.

hatched, but there was no apparent motility of the miracidia. Similarly, concentrations with a pH of 7.6, with the exception of the 0.1% NaCl dilution seemed to effect the release of non-motile miracidia. The control with the buffer solution having a pH of 7.0 resulted in the hatching of the eggs. Some of the eggs which failed to hatch in the high concentrations of NaCl appeared to be plasmolyzed.

At the end of 5 days the potassium phosphate buffer dilutions failed to effect hatching, though the buffer control having a pH of 7.0 did effect hatching in 2 days.

Effect of varying concentrations of KCl in buffered solutions.

The effective pH range, in the presence of KCl, for the liberation of miracidia shifted toward the less acid concentration (Table 3). The data show that 0.7% KCl at a pH of 7 using potassium phosphate buffer effected the escape of the miracidia most quickly. The miracidia liberated in this solution were more actively motile than were those in any of the other solutions used. Some of the eggs hatched in the potassium phosphate buffer control having a pH of 7.0. Here, as in the controls in the previous experiments, the liberated miracidia appeared to be non-motile.

CONCLUSION

It has been possible to effect the hatching of the eggs of *Plagitura salamandra* by means of NaCl and KCl solutions having certain pH ranges at room temperature.

TABLE 3.—*Effect of KCl prepared in Clark and Lubs buffers and in potassium phosphate buffers on the hatching of eggs containing active miracidia at 18°–21° C.*

KCl Conc.	*Clark and Lubs Buffers*					*Potassium Phosphate Buffers*		
	pH 4.0	pH 5.0	pH 7.0	pH 8.0	pH 8.6	pH 5.0	pH 7.0	pH 7.6
0.1%	–	–	–	–	4*	–	–	–
0.2%	–	–	4	–	4	–	–	2 M
0.3%	–	–	3 M**	3	3	–	–	2 M
0.4%	–	–	3 M	3	4	–	–	4
0.5%	–	–	5	3	3 M	–	–	2 M
0.6%	–	–	5	3	4 M	–	–	2 M
0.7%	–	–	4 M	4 M	4 M	–	1 M	4 M
0.8%	–	–	4 M	4 M	4 M	–	3 M	4 M
0.9%	–	–	5	4	4	–	3	4
1.0%	–	–	5	4	4	–	3	4
Controls:								
Buffer only	–	–	2	–	–	–	2	–

* Number refers to days required for eggs to hatch.
** M indicates that some of the miracidia were motile after hatching.

The mechanism utilized in the liberation of the miracidia from the trematode eggs has not yet been determined.

SUMMARY

1. Mature miracidia of *Plagitura salamandra* were hatched within 48 hours in the presence of intestinal tract extract of the host specific snail, *Pseudosuccinea columella;* the addition of NaCl to the extract effected hatching of motile miracidia in 24 hours.

2. Aqueous extracts of 3 species of aquatic snails resulted in the liberation of the mature miracidia only after the addition of NaCl. These miracidia, however, did not exhibit motility.

3. A study of the results obtained from exposing the eggs containing miracidia to buffered concentrations of NaCl and KCl from 0.1%–1.0%, having pH range of 4.0–8.6, permit the following statements:

a. Eggs of *Plagitura salamandra* containing mature miracidia hatched in a range of concentrations of NaCl with pH from 5.0–7.6 and KCl having pH from 7.0–8.6.

b. The released miracidia did not appear motile in all the solutions.

c. Hatching was observed in buffer solutions having a pH of 7.0

ACKNOWLEDGMENT

I wish to express my sincere appreciation to Professor B. D. Reynolds for his many helpful suggestions and the interest he has shown in this study.

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THE OCCURRENCE OF THE COCCIDIAN EIMERIA BUKIDNONENSIS TUBANGUI 1931, IN NIGERIAN CATTLE

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During the course of routine examinations a number of unusual coccidia oöcysts were found in the feces of a Fulani calf from Kagoro, Zaria Province. The parasites were scarce but 60 were recovered for detailed morphological examination and a few were available for sporulation tests. They were subsquently identified as *Eimeria bukidnonensis*. There are relatively few references to this parasite in the literature and as it appears to be the first time the species has been described from Nigeria it is considered desirable to record the findings in detail.

Eimeria bukidnonensis was originally described by Tubangui (1931) from the feces of a bull brought to Manila, from Bukidnon, Mindanao. Twenty-five oöcysts were examined. It was subsequently observed by Yakimov, Gusev, and Rastegaeva (1932) in Usbekistan and by Marčenko (1937) in North Caucasia.

It seems likely that the parasite occurs in Brazil since Yakimov (1936) found it

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TABLE 1.—*Dimensions of* Eimeria bukidnonensis *recorded by various workers*

Measurements in microns	Kagoro oöcysts (present author)	Mindanao oöcysts (Tubangui, 1931)	Alabama oöcysts (Christensen, 1941)	New York oöcysts (Christensen, 1938)
Mean length	44.0	48.6	36.6	..
Range in lengths	41.2–47.9	46.8–50.4	33–41	32–41
Mean width	32.1	35.4	26.7	..
Range in width	29.9–34.0	33.3–37.8	24–28	24–30
Mean shape index	1.37	1.37	1.37	..
Range in shape indices	1.27–1.45	..	1.25–1.56	..
No. of oöcysts measured	60	25	80	10

in 1 of 49 bovines imported from Sao Paulo to Leningrad. The first records of its recognition in North America appear to have been made by Christensen (1938) and Baker (1938), 10 oöcysts being examined from the same occurrence of the species. The next report of its occurrence known to the present author is by Christensen (1941) in which he described 80 oöcysts found in the feces of 3 bovines in Alabama.

MATERIALS AND METHODS

In the present examinations the oöcysts were removed from the rectum manually and were placed with ice in a thermos flask for transportation to the laboratory. They remained on ice until required for examination. Oöcysts were recovered by passing a watery suspension of feces through a fine-mesh sieve and the filtrate was concentrated and washed by centrifuging. Concentrated sugar solution was added to the final deposit and the mixture was again centrifuged to bring the oöcysts to the surface. A drop was removed by means of a platinum loop and transferred to a cover slip which was lowered onto a hollow ground slide. In this way oöcysts floated free under the surface of the cover slip and were therefore not subjected to any pressure liable to cause distortion. Examinations and measurements were made under oil immersion by means of an ocular micrometer.

Sporulation was attempted in 2% potassium dichromate in a shallow watch glass at room temperature and sporulating oöcysts were examined daily.

FINDINGS

The oöcysts were readily distinguished from other coccidia in the preparations by their striking yellowish-brown color. They were pyriform and had a thick wall of approximately 2 microns in transverse section. The wall was of a darker color than the remainder of the oöcysts and invariably showed marked radial striations.

The micropyle was distinct and varied from 3.5 to 4.0 microns in diameter. The

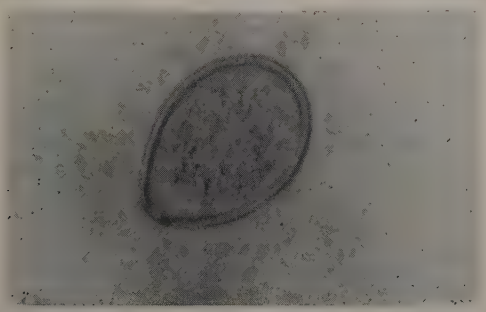

FIGURE 1. Unsporulated oöcyst of *Eimeria bukidnonensis,* approximately 32 microns wide. (Under oil immersion.)

other dimensions are shown in Table 1 together with the measurements recorded by the other workers referred to in this paper for comparison.

Only a few oöcysts were available for sporulation. Sporozoites in an advanced state of development were observed on the 17th day but, unfortunately, they were lost and no further material was obtainable. It is interesting to note that Baker (1938) observed the sporulation period to be 24–27 days while Christensen obtained complete sporulation in 4 to 7 days.

Since these examinations were made oöcysts morphologically similar to the above have been seen in cattle feces submitted to this laboratory from widely scattered areas of Northern Nigeria. Unfortunately, all these samples were stored during transit in formalin and it was therefore impossible to carry out sporulation tests.

ACKNOWLEDGEMENTS

I am indebted to the Inspector-General of Animal Health Services, Nigeria, and to the Assistant Director of Laboratory Services for permission to publish this note and to Mr. R. A. O. Shonekan, Laboratory Technician, for technical assistance.

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CAMBARINCOLA GRACILIS SP. NOV., A BRANCHIOBDELLID OLIGOCHAETE COMMENSAL ON WESTERN CRAYFISHES

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While studying the branchiobdellids from western crayfishes, an undescribed species of *Cambarincola* was found on crayfishes collected at a number of localities. This new species, for which the name *Cambarincola gracilis* is proposed, is similar to both *C. macrodonta* Ellis, 1912 and *C. inversa* Ellis, 1919, but may be differentiated from these on the basis of the structure of the head, jaws, and accessory sperm tube.

I wish to express my appreciation to Dr. Eugene N. Kozloff of Lewis and Clark College for his guidance and encouragement throughout the progress of this study. Gratitude is also extended to Dr. Fenner A. Chace, Jr. for the loan of paratypes of *Cambarincola inversa* in the United States National Museum.

METHODS

The worms were taken from crayfish which had been preserved in higher grades of ethyl and isopropyl alcohol. The most satisfactory whole mounts were prepared

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by progressive staining with Delafield's alum hematoxylin. Unstained specimens and specimens stained with borax carmine were also used, but these were in general less suitable for morphological study. Sagittal and transverse serial sections of worms embedded in paraffin were stained with Delafield's hematoxylin.

Cambarincola gracilis sp. nov.

The general outline and external appearance of this species (Fig. 1) is quite similar to that of *C. inversa,* described by Ellis (1919). The body is elongate and terete, becoming gradually wider from segment I to segment VI, the widest segment. The posterior segments taper rapidly to the terminal acetabulum. The length of sexually mature worms ranges from about 2 mm. to about 4 mm. The width, at segment VI, of a specimen about 2 mm. long is approximately 0.4 mm.

The head is approximately as long as the first 3 body segments combined. Its width is slightly greater than that of segment I. The lips are separated from the head by a constriction, and the jaws are located in this narrowed region. There are 2 lips, both entire, the upper being slightly longer than the lower. The pharynx has 2 diverticula, one dorsal, the other ventral. The dorsal diverticulum is slightly anterior to the ventral one.

The jaws are yellow brown. The dorsal jaw (Figs. 2a, 2b) bears 1 large median tooth and 2 weakly developed tubercles on the dental ridge. The ventral jaw (Figs. 2c, 2d) has 2 teeth which are smaller than the single tooth of the dorsal jaw, and 2 small, sharp tubercles on the dental ridge. The jaws of *C. gracilis* do not have the excavated and somewhat triangular base described for the jaws of *C. macrodonta* and *C. inversa.* The base, as seen in dorsal or ventral view, is rounded.

The reproductive organs are characteristic of the genus. There are 2 pairs of testes, a pair in segment V, the other pair in segment VI. The spermatheca is not bifid, and opens by a prominent pore on the ventral side of segment V. The bursa in segment VI (Figs. 3, 4) is conspicuous and eversible. The accessory sperm tube enters the genital atrium near the point where the latter joins the duct of the bursa. The vasa deferentia leading from the testes in segments V and VI enter the anterior portion of the genital atrium. There is a pair of ovaries in segment VII.

A list of the host species of crayfish together with the different locations from which *Cambarincola gracilis* was taken follows. *Pacifastacus klamathensis* (Stimpson): **Washington**—Walla Walla Co. (campus of Whitman College, Walla Walla), Clallam Co. (tributary of Elwha River); **Oregon**—Multnomah Co. (Crystal Springs Creek, Portland), Clackamas Co. (Tryon Creek, near Oswego), Lincoln Co. (Yachats River 1 mile east of Yachats); **California**—Siskiyou Co. (Klamath River 1 mile west of mouth of Ash Creek; same river 2 miles east of Hornbrook); *P. trowbridgii* (Stimpson): **British Columbia**—Burdette Creek, (near Burnaby), Colquitz River (near Victoria); *P. gambelii* (Girard): **Oregon**—Harney Co. (near OO Ranch Corral, Malheur National Wildlife Refuge).

The holotype, from *Pacifastacus klamathensis* collected by Dr. A. G. Rempel on the campus of Whitman College, Walla Walla County, Washington, has been deposited in the United States National Museum (U.S.N.M. No. 26110). The two paratypes (U.S.N.M. Nos. 26111, 26112) have been selected from the specimens taken from *P. klamathensis* collected by Mr. Harold Wolf in the Klamath River 1 mile west of the mouth of Ash Creek, Siskiyou County, California and from *P. trow-*

bridgii collected by G. Clifford Carl in Burdette Creek, near Burnaby, British Columbia.

DISCUSSION

Because *Cambarincola gracilis* is similar to *C. inversa* in general form, it is possible that some specimens of *C. gracilis* may have been referred to *C. inversa* by earlier authors. Goodnight (1940) recorded *C. inversa* from a number of stations in Oregon, Washington, and Idaho. Although I have examined crayfishes from widely scattered localities in northern California, Oregon, Washington, and British Columbia, I did not find branchiobdellids conspecific with the paratypes of *C. inversa.*

Cambarincola gracilis may be differentiated from *C. inversa* by the structure of its jaws and the organization of the accessory sperm tube. In *C. gracilis* the base of the jaws is rounded, whereas in *C. inversa* the base is excavated. In *C. inversa* there is a smaller tooth on either side of the large median tooth of the dorsal jaw, but in *C. gracilis* these lateral teeth are lacking. The ventral jaw of *C. gracilis* also lacks the lateral teeth present on the ventral jaw of *C. inversa.* In dorsal or ventral view, the jaws of *C. inversa* are somewhat triangular in outline, and are widest anteriorly.

The accessory sperm tube of *C. gracilis* is very unlike that of *C. inversa,* and more closely resembles the accessory sperm tube of *C. macrondonta,* described by Ellis (1912). It appears to be a relatively undeveloped structure, closely applied to the genital atrium and opening into the atrium at the point where the latter joins the duct of the bursa. In *C. inversa* the accessory sperm tube is large, and is apparently entirely free of the atrium except where it opens into the latter.

SUMMARY

Cambarincola gracilis sp. nov. is described from crayfishes referable to *Pacifastacus klamathensis, P. trowbridgii,* and *P. gambelii* collected at a number of localities in northern California, Oregon, Washington, and British Columbia. *C. gracilis* appears to be closely related to *C. inversa* Ellis and *C. macrodonta* Ellis.

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EXPLANATION OF PLATE

Cambarincola gracilis sp. nov.

The figures have been prepared with the aid of a camera lucida, and with the exception of Figs. 2b and 2d are oriented in such a way that the dorsal aspect is nearest the top of the page.

Abbreviations used: ast, accessory sperm tube; b, bursa; d, duct of bursa; ga, genital atrium; vd, vas deferens.

FIG. 1. Entire worm; lateral view from left.

FIG. 2a. Dorsal jaw; lateral view from left.

FIG. 2b. Dorsal jaw; dorsal view.

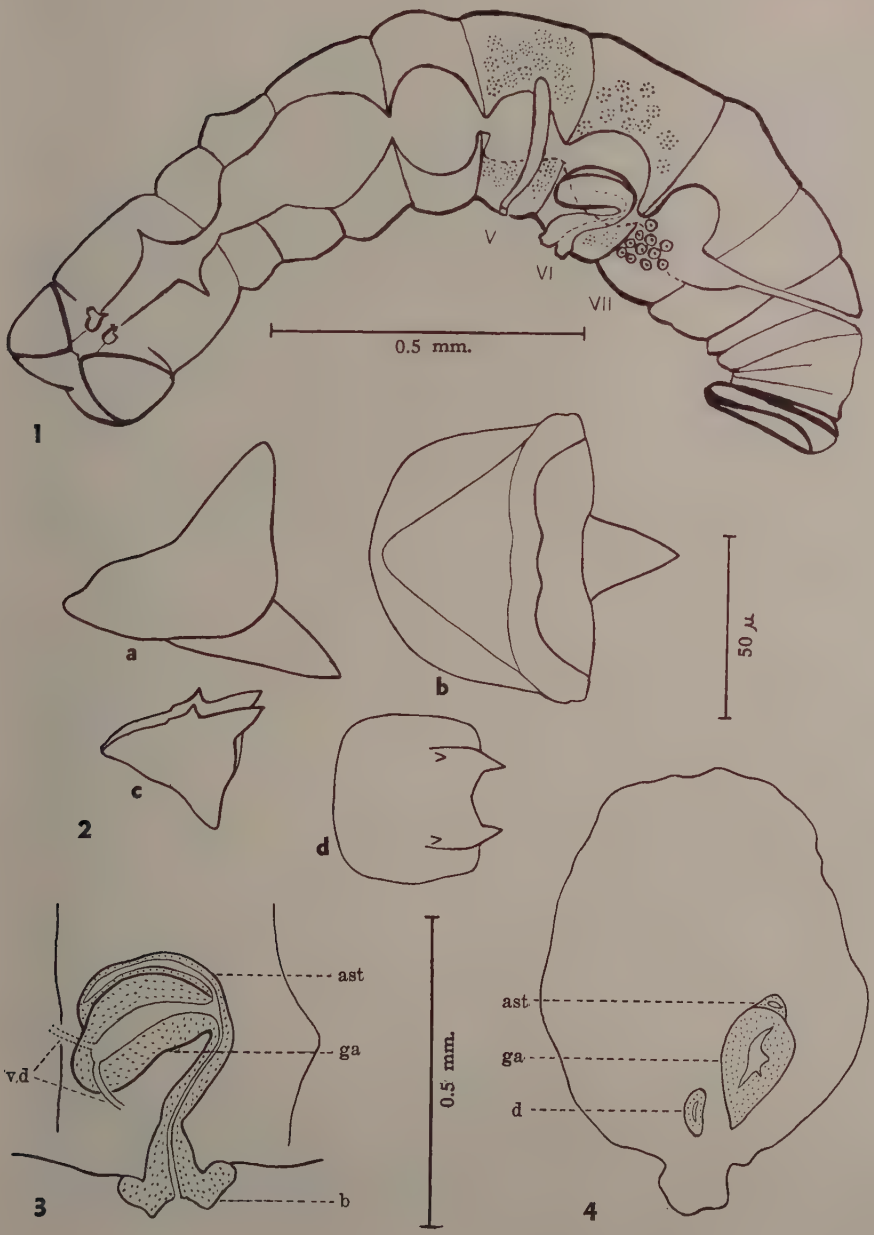
FIG. 2c. Ventral jaw; lateral view from left.

FIG. 3. Male reproductive organs in segment VI (testes omitted); semidiagrammatic, reconstructed from sagittal serial sections.

FIG. 4. Male reproductive organs in transverse section through posterior half of segment VI; semidiagrammatic.

FIG. 2d. Ventral jaw; dorsal view.

PLATE I



VENTRIDENS LIGERA, A NEW FIRST INTERMEDIATE HOST OF *PANOPISTUS PRICEI* SINITSIN, 1931 (TREMATODA: BRACHYLAIMATIDAE)[1]

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In at least 9 of the experimentally determined life cycles of the BRACHYLAIMATINAE, from 3 to 9 species of mollusks have been recorded as second intermediate hosts of single brachylaimatid species. In the literature of the BRACHYLAIMATINAE reviewed a single brachylaimatid, viz. *Panopistus pricei,* has been reported to utilize more than 1 molluscan species as the first intermediate host. In this case 2 land snails, *Zonitoides arboreus* and *Agriolimax agrestis,* were reported as primary hosts.

Sinitsin (1931) briefly described a metacercaria found in only 1 of 138 specimens of *Gastrodonta suppressa* (now known as *Ventridens suppressus*). This metacercaria he considered to be the larval form of an adult brachylaimatid parasitic in the small intestine of the short-tailed shrew, *Blarina brevicauda* (Say). Krull (1935), believing that Sinitsin's evidence was inconclusive, determined experimentally that *Zonitoides arboreus* served as a first as well as a second intermediate host of *P. pricei.* Reynolds (1938), without controlled feeding experiments, described cercarial and metacercarial stages of this brachylaimatid from the slug, *Agriolimax agrestis,* in Virginia.

OBSERVATIONS

In a survey of the land snails of the Ann Arbor area during 1950–1952, metacercariae believed to be *P. Pricei,* were recovered from the heart and pericardial cavity of *Ventridens ligera, Stenotrema monodon, Zonitoides nitidus,* and a slug, *Deroceras laeve.* Of 3844 *Ventridens ligera* collected, 3 were shedding blunt-tailed brachylaimatid cercariae and about 50 percent of the snails were parasitized with brachylaimatid metacercariae. The location of the genital pore (behind the posterior testis) suggested that the metacercariae removed from the heart and pericardial cavity belonged to the genus *Panopistus* Sinitsin, 1931.

Sinitsin's original metacercaria of *P. pricei* from *Ventridens suppressus,* slide #28360, U. S. Nat'l. Mus. Helm. Coll., was loaned to the writer by Dr. E. W. Price for comparison with the metacercariae obtained from *Ventridens ligera* and other land snails, collected for the present study. An examination of the slide showed that 1 of 2 metacercariae was identical with the metacercariae from *Ventridens ligera, Stenotrema monodon, Zonitoides nitidus,* and *Deroceras laeve.* This suggested that Sinitsin correctly identified the metacercaria from *Ventridens suppressus* as a larval form of *P. pricei.* A second metacercaria on this slide, unidentified on the label, has been identified by the writer as *Entosiphonus thompsoni,* also described by Sinitsin (1931). Since multiple infections of brachylaimatids are quite common in land snails, *P. pricei* and *Entosiphonus thompsoni* probably were

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[1] Appreciation is expressed to Dr. A. E. Woodhead, Department of Zoology, under whom this work was done. Contribution from the Department of Zoology, University of Michigan.

recovered from the same snail. I have frequently observed *P. pricei* associated with other species of brachylaimatids within the same snail host.

EXPERIMENTS AND RESULTS

To verify the identity of this brachylaimatid, the life cycle was carried out under laboratory conditions. Of 86 short-tailed shrews collected from areas known to have snails infected with brachylaimatid metacercariae, 42 were infected with mature and immature adults of *P. pricei*. Eggs from mature worms were then fed to laboratory-raised *Ventridens ligera, Stenotrema monodon, Zonitoides nitidus,* and *Deroceras laeve*. Branching sporocysts developed only in *Ventridens ligera*, producing cercariae identical with the cercariae observed in naturally-infected *Ventridens ligera*. Cercariae were produced in 44–50 days following exposure of the snails to the eggs of *P. pricei*. Measurements in millimeters of various structures of 25 cercariae from *Ventridens ligera* (fixed in hot 10 percent formalin) were as follows: body length, including tail 0.184 to 0.272 (av. 0.225); width 0.067 to 0.102 (av. 0.082); oral sucker 0.028 to 0.058 (av. 0.049) by 0.046 to 0.058 (av. 0.052); pharynx 0.018 to 0.025 (av. 0.021) by 0.018 to 0.023 (av. 0.022); acetabulum 0.037 to 0.051 (av. 0.045) by 0.041 to 0.058 (av. 0.050). These values are essentially within the range of dimensions listed by Krull for the cercariae from *Zonitoides arboreus*. The number (7 pairs) and arrangement of the flame cells and the general morphology of the cercaria from *Ventridens ligera* agree with similar data for the cercaria from *Zonitoides arboreus*, as reported by Krull.

Cercariae from laboratory-raised *Ventridens ligera* were transferred to laboratory-raised second intermediate hosts. Metacercariae, identical with those in natural infections, were recovered from the laboratory-raised snails 75–90 days following exposure of the snails to cercariae. Measurements in millimeters of various structures of 25 metacercariae from *Ventridens ligera* (fixed in hot 10 percent formalin, stained and mounted) were as follows: body length 0.775 to 0.857 (av. 0.825); width 0.272 to 0.340 (av. 0.311); oral sucker 0.136 to 0.197 (av. 0.185) by 0.156 to 0.197 (av. 0.166); pharynx 0.068 to 0.095 (av. 0.083) by 0.082 to 0.102 (av. 0.092); acetabulum 0.136 to 0.190 (av. 0.171) by 0.143 to 0.190 (av. 0.168); anterior testis 0.054 to 0.075 (av. 0.063) by 0.041 to 0.061 (av. 0.052); posterior testis 0.048 to 0.068 (av. 0.056) by 0.034 to 0.045 (av. 0.042); ovary 0.034 to 0.045 (av. 0.040) by 0.034 to 0.048 (av. 0.044). Several of the foregoing dimensions do not agree with those listed by Krull for the metacercariae of *P. pricei* from *Zonitoides arboreus*. However, differences may be due to the method of fixation, state of contraction, or possibly to the age of the metacercariae.

The time required for the development of the intramolluscan stages in *Ventridens ligera* agrees essentially with the time reported by Krull for similar stages of *P. pricei* from *Zonitoides arboreus*.

Because of the difficulty of raising the short-tailed shrew in the laboratory, 75–90 day old metacercariae of *P. pricei* were fed to 3 albino rats, 3 albino mice, 2 domestic rabbits, and 1 young cottontail rabbit. Adults of *Panopistus* were never recovered from these hosts.

SUMMARY

This study indicates that *Ventridens ligera* is a new, and the third recorded, first intermediate host of *P. pricei*, a brachylaimatid trematode parasitic in the small

intestine of the short-tailed shrew. *Zonitoides arboreus* and the land slug, *Agriolimax agrestis,* had been reported previously as first intermediate hosts of this brachylaimatid. Further, Sinitsin apparently recognized and correctly identified the metacercaria from the pericardial chamber of *Ventridens suppressus* as an intramolluscan stage of this parasite, and Krull demonstrated that *Zonitoides arboreus* may also serve as an experimental second intermediate host. To these the present study adds 4 previously unreported snail species, *Zonitoides nitidus, Stenotrema monodon, Ventridens ligera,* and *Deroceras laeve,* making a total of 6 snail species that serve as second intermediate hosts.

The utilization of 3 species of land snails as primary hosts by *Panopistus pricei* indicates a lack of specificity for the first intermedate host, as well as for the second intermediate host.

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RESISTANCE OF A PYRIMETHAMINE–RESISTANT STRAIN OF *PLASMODIUM GALLINACEUM* TO CERTAIN 2,4–DIAMINOPYRIMIDINES AND RELATED COMPOUNDS

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Strains of several species of malaria made resistant to pyrimethamine, 2,4-diamino-5-(*p*-chlorophenyl)-6-ethylpyrimidine, have been found to be resistant to chlorguanide (*Plasmodium cynomolgi,* Schmidt and Genther, 1952; *P. vivax,* Hernandez, Myatt, Coatney and Jeffery, 1953; *P. berghei,* Thurston, 1953). Strains of parasites resistant to chlorguanide sometimes are (Robertson, Davey and Fairley, 1952; Jaswant Singh, Ray, Basu and Nair, 1952; Jones, 1953) and sometimes are not resistant to pyrimethamine (Rollo, 1951; Wilson and Edeson, 1953). We found that a strain of *P. gallinaceum* resistant to chlorguanide was not resistant to 2,4-diamino-5-(*p*-chlorophenoxy)-6-methylpyrimidine (Greenberg and Richeson, 1951). Schmidt (personal communication) observed a strain of *P. cynomolgi* resistant to pyrimethamine that was not resistant to the 5-*p*-chlorophenoxy analogue.

There are many reasons for believing that the modes of action of 2,4-diaminopyrimidines and chlorguanide are similar. The failure to obtain cross-resistance would imply that resistance is associated with changes in the parasite involving particular portions of the molecule. To locate the portion of the molecule involved, a limited series of diaminopyrimidines, a diaminotriazine, and chlorguanide were tested against a strain of *P. gallinaceum* made resistant to pyrimethamine.

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MATERIALS AND METHODS

The pyrimethamine-resistant strain of *P. gallinaceum* used in these experiments was obtained by the usual procedure of exposing the parasite to increasing amounts of the drug over a period of time. The drug was administered at minimal effective doses, twice daily for 4 days beginning 4 to 5 hours before inoculation of parasites. When the mean parasitemia of the treated chicks, examined on day four, significantly exceeded 25 per cent of the controls, the dosage of drug administered in the next passage was doubled. A three-day drug-free period usually intervened between passages. Treatment was discontinued when it was found that 64 times as much pyrimethamine was required to suppress the resistant strain as was required for the parent strain. At the time the present experiments were carried out the resistant strain had not been exposed to drug for about 6 months.

Week-old New Hampshire Red chicks, weighing 45 to 60 grams, served as hosts. The methods of inoculating parasites, determination of parasitemia, and criteria for minimal effective dose have been described elsewhere (Coatney, Cooper, Eddy and Greenberg, 1953). Briefly, the chicks were inoculated intravenously with 16 million parasitized erythrocytes. They were treated twice daily with the test compounds for 4 days beginning 4 to 5 hours before inoculation. Parasitemia was determined on the morning of the fifth day. The minimum effective dose of a compound is that amount which reduced the parasitemia of the treated chicks to at least one-fourth of that of the untreated controls. Five chicks were used for each dosage regimen, and for each series 10 chicks served as untreated controls.

All of the pyrimidines except the diacetyl derivative of pyrimethamine were kindly furnished by Burroughs Wellcome and Company (U.S.A.), Inc., chlorguanide and the triazine by Imperial Chemical Industries. The diacetyl derivative of pyrimethamine was synthesized by one of us (H.W.B.).

RESULTS

The results are summarized in the table. The treated strain was 64-fold resistant to pyrimethamine and to the diacetyl derivative of pyrimethamine. It was 512-fold resistant to the 6-amyl analogue (DR 16,093) of pyrimethamine; it was

TABLE 1.—*The effect of diaminopyrimidines, chlorguanide, and a diaminotriazine on a pyrimethamine-resistant strain of* Plasmodium gallinaceum

Compound	Minimum effective dose (mg./gm.)		
	Parent strain (P)	Resistant strain (R)	R/P
DR 16,056	0.00004	0.00256	64
DR 16,311	0.00008	0.00128	16
DR 16,093	0.0000625	0.032	512
DR 16,279	0.00004	0.0025	64
DR 16,071	0.001	0.008	8
DR 16,092	0.001	0.002	2
DR 15,787	0.0078	0.0156	2
SN 12,837	0.002	0.032	16
DR 16,182	0.00002	0.00016	8

DR 16,056—Pyrimethamine : 2,4-diamino-5-(*p*-chlorophenyl)-6-ethylpyrimidine
DR 16,311—2,4-Diamino-5-(3′,4′-dichlorophenyl)-6-methylpyrimidine
DR 16,093—2,4-Diamino-5-(*p*-chlorophenyl)-6-amylpyrimidine
DR 16,279—2,4-Diacetylamino-5-(*p*-chlorophenyl)-6-ethylpyrimidine
DR 16,071—2,4-Diamino-5-(*p*-chlorobenzyl)-6-methylpyrimidine
DR 16,092—2,4-Diamino-5-(*p*-nitrobenzyl)-6-methylpyrimidine
DR 15,787—2,4-Diamino-5-(*p*-chlorophenoxy)-6-methylpyrimidine
SN 12,837—Chlorguanide : N_1-(*p*-chlorophenyl)-N_5-isopropylbiguanide
DR 16,182—4,6-Diamino-1-(3′,4′-dichlorophenyl)-1,2-dihydro-2,2-dimethyl-1,3,5-triazine

relatively less resistant to the 6-methyl analogue (DR 16,311) though the reduction in activity might be attributable to the second chlorine on the 5-phenyl ring.

The strain was relatively less resistant to pyrimidines in which the linkage between the 5-phenyl ring and the pyrimidine nucleus was altered. The strain was only 8-fold resistant to the 5-benzyl-6-methyl analogue (DR 16,071) of pyrimethamine; and when the chlorine of this compound was replaced with a nitro group (DR 16,092) resistance almost disappeared. The pyrimethamine-resistant strain was almost as sensitive as the parent strain to the 5-(*p*-chlorophenoxy)-6-methyl analogue (DR 15,787) of pyrimethamine.

The pyrimethamine-resistant strain was about 16-fold resistant to chlorguanide (SN 12,837) and about 8-fold resistant to the triazine (DR 16,182) which is reported to be the active metabolic product of the dichlorophenyl analogue of chlorguanide (Crowther and Levi, 1953).

DISCUSSION

It is apparent that resistance to pyrimethamine is not always associated with equal resistance to related compounds. There appears to be a rough correlation between the degree of resistance to a compound and the activity of the compound against the parent strain. Looked at another way, certain changes in the substituents of the pyrimidine molecule produce relatively larger changes in the degree of activity against the parent strain of the parasite than do others. For example, the manner in which the phenyl group is attached to the pyrimidine ring influences antimalarial activity more than changes in the 6-position substituent. The changes in the molecule which appear to make the largest changes in the degree of activity of the compound appear to make the largest differences in the degree of resistance of the pyrimethamine-resistant strain. (The 6-amyl compound, DR 16,093, is an obvious exception.) This suggests that resistance is related to focal points in the molecule with, perhaps, the manner of attachment of the phenyl group to the pyrimidine ring being the most important. The degree of resistance becomes increasingly smaller as one proceeds from the 5-phenyl compounds, through the 5-benzyl compounds, to the 5-phenoxy compound. The lower degree of resistance of the triazine may be considered to be related to the fact that the phenyl group is attached through a ring nitrogen. The tentative relationships suggest that development of resistance in the organism to these pyrimidines may be accomplished by changes in an "acceptor" molecule in the parasite, rather than by the development of a metabolic shunt mechanism. It is also likely that, if such changes occur, they do so in small steps, since the degree of resistance to pyrimethamine presumably can be maintained at any point by withdrawing the parasite from exposure to the drug.

These tentative conclusions point to several questions, among them being: (1) do the changes which produce resistance always occur in the same direction or in the same order? (2) do all strains of a parasite which have acquired the same degree of resistance to one diaminopyrimidine respond the same to related compounds?

There are many unexplained facts about resistance and cross-resistance. Parasites which are resistant to pyrimethamine are usually somewhat resistant to chlorguanide (see introduction); but the converse is not always true. (The chlor-

guanide-resistant strain maintained in this Laboratory is not significantly resistant to pyrimethamine.) Our pyrimethamine-resistant strain, as reported in this paper, is 16-fold resistant to chlorguanide. Neither of these strains is significantly resistant to the 5-(*p*-chlorophenoxy) pyrimidine (DR 15,787). And, finally, we have not been able to develop a strain resistant to the last compound.

SUMMARY

Seven diaminopyrimidines (including pyrimethamine), chlorguanide, and a diaminotriazine were tested against a pyrimethamine-resistant strain of *Plasmodium gallinaceum*. The strain was 64-fold resistant to pyrimethamine. It showed relatively little resistance (2-fold) to some chemically related compounds and marked cross-resistance (64- to 512-fold) to others. It was about 16-fold resistant to chlorguanide and 8-fold resistant to a diaminotriazine related to chlorguanide.

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FURTHER STUDIES ON THE HYBRIDIZATION OF STRAINS OF *PLASMODIUM GALLINACEUM*

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The BI strain of *Plasmodium gallinaceum* does not produce normal erythrocytic parasites when passaged through the mosquito (Haas, Wilcox, Ewing and Coleman, 1948; Trembley, Greenberg and Coatney, 1951; and Greenberg, Trembley and Coat-

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[1] Laboratory of Tropical Diseases.

ney, 1953). The chicks die of late exoerythrocytic parasites, with a few, non-pigmented parasites detectable in the erythrocytes just prior to death. The strain can normally undergo only one transfer through the mosquito without the intervention of the investigator. If the latter can keep the chicks alive by therapy (Greenberg *et al.*, 1953) or keep the parasite alive by the serial subinoculation of blood (Lewert, 1948, 1950; Haas *et al.*, 1948), the strain eventually regains its ability to produce normal erythrocytic parasites and is again infectious to the mosquito. The M strain, on the contrary, is benign and produces no detectable late exoerythrocytic forms when passaged through the mosquito (Hass *et al.*, 1948; Trembley, Greenberg and Coatney, 1951a). Dr. Clay G. Huff was kind enough to examine tissues of chicks infected with the M strain parasites and found late tissue forms in serial sections of brain and other tissues. We have not observed such forms in routine examinations of brain smears made at autopsy; and, since the mortality rate of this strain approaches zero percent, such forms obviously do not contribute to the death of the chicks. Conversely, the BI strain when mosquito-passaged produces abundant phanerozoites, readily detectable in brain smears and causing a mortality of 100 percent.

In an earlier experiment (Greenberg and Trembley, 1953) chicks were inoculated intravenously with equal numbers of erythrocytic forms of both strains of the parasite. Mosquitoes were fed on these chicks, and the sporozoites from these mosquitoes were used to infect other chicks. The resulting infections (F_1) in these chicks had both erythrocytic and exoerythrocytic parasites, indicating that the characteristics of both strains were present. Mosquitoes were fed on the chicks harboring the F_1 infections, and the sporozoites from these mosquitoes produced infections (F_2) in no way distinguishable from the parent M strain infections. The F_2 infections were benign and devoid of late tissue forms. This pattern of infection persisted through another mosquito passage (F_3).

The following possible explanations were offered to explain these results: (1) no hybridization occurred; (2) hybridization occurred but the genes producing the BI strain characteristics were dominant and the hybrids behaved as the parent BI strain, i.e., only one mosquito passage was possible without the intervention of the investigator; (3) the hybrids all behaved as the parent M strain because of a cytoplasmically inherited factor which, for example, limited reproduction in the fixed tissue.

It was felt that more information could be obtained if the strains were exposed to the possibility of two successive hybridizations. This could be done conveniently by treating the F_1 infections with pyrimethamine, which prevents death from tissue forms (Greenberg, Trembley and Coatney, 1953). When chicks infected with BI strain sporozoites only were treated at about day 7 after inoculation, normal erythrocytic parasites were detected after day 18, and the strain was infectious to mosquitoes after day 20. When sporozoite-induced M strain infections were treated similarly, the course of the infection after treatment was about the same as that of the BI strain, i.e., the strain was infectious after day 20 (unpublished data). In an attempt to bring about a second hybridization (?), chicks carrying the F_1 generation of hybrid (?), M × BI, infections were treated with pyrimethamine on or about the seventh day after inoculation, and mosquitoes were allowed to feed on or after day 20.

MATERIALS AND METHODS

Week-old New Hampshire Red chicks weighing 45 to 60 grams were used. The vector was laboratory-reared *Aedes aegypti*. Two strains of *P. gallinaceum*, M and

BI, were used; their principal characteristics are described in the first paragraph of this paper. One mosquito equivalent of sporozoites was inoculated according to the method recommended by Coatney, Cooper and Trembley (1945). The methods of inoculating blood and of making and reading blood smears for parasitemia have been described by Coatney, Cooper, Eddy and Greenberg (1953). Blood smears were read daily or every other day beginning on day 7 after the inoculation of sporozoites. Brain smears of all chicks were made at autopsy and examined for tissue forms of the parasite (Coatney *et al.*, 1945). Pyrimethamine was administered orally by catheter in dilute lactic acid.

RESULTS

Heparinized blood from a donor chick infected by sporozoites with the M strain and from a donor chick infected by blood passage with the BI strain of *P. gallinaceum* was diluted with physiological saline and pooled so that approximately equal numbers of parasites of each strain were present in the preparation (8×10^6 parasitized erythrocytes per 0.1 ml.). Each of 10 chicks was inoculated with 0.1 ml. of this mixture. Of these, 3 chicks were selected, 2 on day 7 and 1 on day 8, each 1 to act as host for feeding a single lot of 200 mosquitoes. Eleven and 12 days later sporozoites from each of the 3 lots of mosquitoes were used to infect 10 chicks for a total of 30. On day 9 of the infection, each chick of the latter group was treated with a single dose (0.016 mg./gm.) of pyrimethamine. Three chicks died of exoerythrocytic infections before treatment, and 4 more chicks died from the same cause one and two days after treatment. When the remaining chicks were killed 24 to 26 days after inoculation, 15 to 17 days after drug, 4 more chicks exhibited rare exoerythrocytic parasites in smears of the brain. Therefore, 11 of the 30 chicks showed evidence of late tissue forms, there being at least 2 from each of the 3 groups.

Mosquitoes were fed on 1 or 2 chicks from each group on the twentieth, twenty-first, and on the twenty-fourth day after inoculation. Ten to 14 days later sporozoites from mosquitoes of each lot were used to infect 10 chicks; a total of 30. Three of these chicks died on day 4 of causes unknown. One chick died on day 15, probably of parasitemia (35 percent on day 14); no tissue forms were detectable. The remaining chicks survived to day 21 when they were killed. All the chicks which survived to day 21 exhibited a normal parasitemia; none had tissue forms in smears of the brain made at autopsy.

DISCUSSION

Following the initial inoculation of infected blood, these experiments were done in triplicate in that 3 independent passage lines were maintained throughout. The results in each of the 3 lines were essentially the same. As in our earlier experiment (Greenberg and Trembley, 1953) the characteristics of the BI strain were present after the first potential hybridization. Seven chicks died of exoerythrocytic infections just before or just after treatment with pyrimethamine and 4 additional chicks exhibited rare tissue forms when autopsied during the fourth week of the infection. None of the chicks used as donors for the F_2 infection had brain forms at autopsy. The ability to produce late tissue forms disappeared after the parasites were allowed a second possible hybridization by passage through mosquitoes. It should be noted that when the BI strain alone was treated similarly the strain characteristics persisted without change through 4 consecutive mosquito transfers (Greenberg *et al.*, 1953).

If there were no hybridization, or if the BI strain characteristics were genetic, dominant or recessive, one would expect that the relative proportions of the 2 strains would remain constant for an indefinite series of passages. This would be true, even if there were a disproportion in the rate of gametocyte production between the 2 strains. The second serial mosquito passage of the mixed-strain infections would be essentially like the first. It is obvious such did not obtain and, therefore, the possibility of no hybridization or of genetic inheritance of BI characteristics can probably be ruled out.

The results do not rule out the possibility of the inheritance of one or more cytoplasmic factors, such as Sonneborn (1949) termed "plasmagenes," and Ephrussi (1953) discussed in a review of nucleo-cytoplasmic relations. Such a factor present in the M strain, for example, might limit the reproduction of the parasite in the fixed tissues. If all M × BI hybrids behaved, because of this cytoplasmic factor, as M strain, the BI strain characteristic of producing late tissue forms would become rarer with each hybridization until it would disappear. The number of hybridizations required for it to disappear would depend partly on chance and partly on the relative abundance of gametocytes produced by the 2 strains. It is our experience that even under the best of conditions it is more difficult to infect mosquitoes with the BI strain than with the M strain, and that gametocytes are extremely rare (about $3/10^4$ parasites) in this strain compared with those in the M strain (almost $4/10^2$ parasites). Therefore, a combination of a cytoplasmically-inherited factor carried by the M strain and a disproportion in gametocyte production is offered as a possible explanation for our results.

Still to be explained are the following facts: (1) the BI strain does, if properly manipulated, produce normal erythrocytic parasites, including gametocytes; (2) the SP strain (Coatney *et al.*, 1945) produces both late tissue forms and erythrocytic parasites when passaged through the mosquito. It is possible that the differences among the strains may be quantitative rather than qualitative or that more than one cytoplasmic factor is involved.

SUMMARY

Two successive mosquito passages of the combined M and BI strains of *Plasmodium gallinaceum* were effected by suppressing tissue forms during the F_1 infection; this allowed for a possible second hybridization of the strains. The original donor chick had been infected with the two strains by blood inoculation, and mosquitoes were allowed to feed on it. Sporozoites from these mosquitoes were used to infect chicks which were treated 9 days after inoculation with pyrimethamine (0.016 mg./gm.). In this F_1 infection gametocytes and some tissue forms were observed, approximately one-third of the chicks exhibiting brain forms either before or shortly after treatment, or at autopsy. Mosquitoes were allowed to feed on these chicks on or after day 20, and the sporozoites were used for a second mosquito passage which resulted in erythrocytic infections only, no late tissue forms being observed when brain smears were examined after the chicks were killed.

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RESEARCH NOTES

EFFECT OF CARBARSONE, CHINIOFON AND HIGH PROTEIN DIET ON TRICHOMONADS IN THE INTESTINE OF THE GOLDEN HAMSTER

Studies in these laboratories show that golden hamsters are often heavily infected with trichomonads and not infrequently harbor *Giardia muris* and *Endamoeba muris*. These hamsters, used in dental caries research, are maintained almost exclusively on a high carbohydrate diet. This kind of diet has been shown by Hegner and Eskridge (1937, J. Parasit. **23:** 105–106) to create favorable conditions for existence of similar parasites in the intestine of rats. Vigorous inbreeding studies with hamsters on a high carbohydrate diet have presented the possibility that vitality of the animals may be diminished and serious consequences result from a disturbance of the balance between host and intestinal protozoa. In order to prepare for such an eventuality it was considered advisable to investigate methods found efficacious in rats to determine their value in removing these parasites from hamsters.

Gabaldon (1936, Am. J. Trop. Med., **16:** 621–639) reported that 150 mg. of carbarsone per kg. of body weight administered orally to rats each day for 5 days eliminated trichomonads. Nelson and Tatum (1938, J. Pharmacol. and Exper. Therap., **63:** 122–142) claimed that chiniofon acted as a well tolerated trichomoniacidal drug in rats. Hegner and Eskridge (1937, J. Parasit., **23:** 105–106) stated that *Endamoeba muris* is eliminated from rats fed on a high protein diet. Ratcliffe (1928, Am. J. Hyg., **8:** 910–934) concluded that the number of trichomonads in rats fed a high protein diet was inversely proportional to the abundance of proteolytic anaerobic bacteria which were stimulated by protein and (1929, Science, **70:** 286–287) reported that removing young rats at 17 days of age from the mother and keeping them free from contamination, resulted in a protozoa-free colony of rats. This latter procedure would not be practical in a colony of hamsters since trichomonads have been observed in the intestine of 6 day old hamsters in this laboratory.

Trichomonad infection in the intestine of 32 golden hamsters was established by noting these flagellates in the soft feces after feeding sunflower seeds or bread soaked in a saturated solution of magnesium sulfate. These animals were treated as follows. Each of 4 hamsters maintained on diet A (45% wheat flour, 30% whole powdered milk, 5% alfalfa, 2% dried yeast, 2% liver powder, 14% corn starch and 2% Wesson's modification of the Osborne-Mendel salt mixture (1932, Science, **75:** 339–340), minus the sodium fluoride was given 15.6 mgs. of carbarsone in 0.5% sodium bicarbonate solution per day for 21 days by introducing the drug into the stomach per os by means of a small syringe and polyethylene capillary tubing (1 mm. bore). Each of 4 others on diet A was given 72.8 mgs. of chiniofon in water per day for 21 days using the same method of administration. Four others on diet B (Dog pellets—Millers Eaties 24% protein—carrots and lettuce) each received 16.25 mgs. of carbarsone in 0.5 per cent sodium

bicarbonate solution per day in their drinking water for 21 days. Similarly, 78.0 mgs. of chiniofon was added to the drinking water of each of 4 other hamsters on diet B each day for 21 days. A high protein diet consisting of 45% ground beef liver, 42% ground beef muscle, 11% lard and 2% calcium carbonate was fed daily for 21 days to 8 other trichomonad infected hamsters. Two groups of 4 hamsters each were used as controls. During the experimental period (21 days) one group was kept on diet A and the other group on diet B.

Seven days after the experiment was started microscopic examination of feces from all animals following the feeding of sunflower seeds or bread soaked in a saturated solution of magnesium sulfate revealed 1. absence of trichomonads in hamsters fed the high protein diet, 2. diminished numbers of trichomonads in hamsters administered carbarsone, 3. large numbers of trichomonads in hamsters given chiniofon and 4. large numbers of trichomonads in all control animals. One week later laparotomies (cecum) performed on one animal from each of the groupings revealed similar findings. Autopsies of all animals at the close of the experiment confirmed the above-mentioned findings. All observations were made of fecal smears prepared by thoroughly mixing approximately one cu. mm. of feces with one drop of physiological saline (0.85% NaCl) and adding a No. 1 cover slip. Using a 15× ocular and 10× objective lens, 10 fields were examined. When 5 or more fields of a smear were observed to be heavily crowded with trichomonads the term "large numbers" was employed to denote the degree of parasitism by this flagellate. Samples showing less than 5 heavily crowded fields were designated by the term "diminished numbers." Absence of trichomonads in animals was concluded upon failure to demonstrate a single trichomonad in separate smears from feces and subsequently in smears from various regions of the intestine at autopsy. A gain in weight was recorded for most animals for the duration of the experiment. Two of the 4 control hamsters on diet A and 3 of the 8 hamsters that received chiniofon suffered a slight loss in weight. *Giardia muris* and *Endamoeba muris* observed at the beginning of the experiment in each of the 8 hamsters placed on the high protein diet were not demonstrable in cecal contents obtained by laparotomy or by examination of all intestinal contents at autopsy.

This work was supported by grants from the University of Rochester and the Medical Research and Development Board, Office of the Surgeon-General, Department of the Army, under Contract No. DA-49-007-MD-495.—WAYNE W. WANTLAND, *Department of Biology, Illinois Wesleyan University, Bloomington, Illinois, and* ERLING JOHANSEN, *Division of Dental Research and Department of Pathology, School of Medicine and Dentistry, University of Rochester, Rochester, N. Y.*

MULTIPLE REPRODUCTIVE ORGANS IN *ASCARIS LUMBRICOIDES*

Recently Monaco and Mizelle (1953, J. Parasitology, **39**: 222) published a brief note on the occurrence of duplicated uteri in *Ascaris lumbricoides* var. *suum*.

A single similar case has now been observed during an analytical and metabolical study of the ovaries of *A. lumbricoides* var. *suum* in which over 500 worms were dissected. In this instance three complete branches of the reproductive organs (ovary, oviduct and uterus) were observed. Two of the uterine branches ramified in the usual manner, while the third branch was joined close to the base of one of these uterine branches.

Monaco and Mizelle gave no details of the branching in their note, duplication of uteri alone being discussed. Accordingly, it is impossible to make a comparison of their observations with mine. However, the present instance of multiple reproductive organs, together with the 3 other cases where multiple uteri were reported (see Monaco and Mizelle, 1953), indicate that multiple reproductive organs occur in *A. lumbricoides* from different localities as a variant from the normal type with two branches of the reproductive organs.—JOHN K. POLLAK, *Institute of Parasitology, McGill University,* (17) *Macdonald College, Province of Quebec, Canada.*

THE LIFE CYCLE OF *NEMATOSPIROIDES DUBIUS* BAYLIS (NEMATODA: HELIGMOSOMIDAE).

Previously only two life cycles have been described for heligmosomids; by Yokogawa (1922, Parasitology **14**: 127–166) for *Nippostrongylus muris,* and Schwartz and Alicata (1935, J. Wash. Acad. Sc. **25**: 128–146) for *Longistriata musculi.* The life cycle for *Nematospiroides dubius* has been determined and is here described. *N. dubius* was collected by Spurlock (1943, J. Parasitol. **29**: 303–311) from feral *Mus musculus,* and by the writer from *Peromyscus maniculatus gambelii* near Davis, California in 1950. The latter constitutes a new host record.

The eggs, measuring 70–84 microns long by 37–53 microns wide, are passed in the feces of the host in the 8–16 cell stage, and become fully embryonated 8–10 hours before hatching. At temperatures of 23–28° C. hatching occurs approximately 26 hours after passing from the host, and only in fecal material which has been kept moist.

The first stage larvae measure 300–600 microns long. Three rudimentary lips are visible around the buccal orifice. The esophagus is oxyuroid in form. The first ecdysis starts 48 hours after hatching and at this time the first stage skin can be seen loosening from both ends of the larva. Ecdysis is not complete outside the host, the first stage skin being retained as a sheath for the infective larva.

The free-living larval stages require 4–6 days to become infective larvae. At this time the latter proceed to the edge of the culture dish and assume a vertical stance using the tail as a foot. They remain motionless for long periods. Mechanical vibration, air currents and heat stimulate activity. The infective larvae measure 480–563 microns long.

Entry into the host is by ingestion, since tests indicate that larvae are unable to penetrate the skin. Upon ingestion the larvae lose their sheaths, and 24 hours later are found in the small intestine closely associated with the mucosa. At this time they measure 376–540 microns long.

From 24–48 hours following ingestion the larvae penetrate the intestinal mucosa and come to lie close to the longitudinal muscle layer of the gut wall. The worm foci are grossly visible as seen from the outside of the intestine. The first evidence of an approaching moult is seen 48 hours after infection. Ninety-six hours after infection most larvae are in the 2nd parasitic stage as pre-adults.

Rapid growth occurs and a third moult is accomplished during the latter pre-adult stages during the 6–8 days after which the worms regain the intestinal lumen and develop to egg laying maturity. Adults are tightly coiled in a spiral, males measuring 8–10 mm. and females 18–21 mm.

Eggs first appear in the feces 9 days after infection. The cycle from egg to egg takes about 15 days. Eggs are produced up to 8 months after infection. No eggs were produced 9 months after infection in one strain of mice. Variations in the lengths of the pre-patent and the patent periods are induced by host strain and also age of the host.—Frank A. Ehrenford, *Pitman-Moore Co., Indianapolis, Indiana.*

A NEW EFFECTIVE ANTHELMINTIC FOR CANINE *TRICHURIS*

In testing a series of compounds for anthelmintic action, 3-methyl-1-pentyn-3-yl sodium phthalate, (P-403), showed significant anthelmintic activity against canine *Trichuris*. The activity of this compound is specific for *Trichuris* in oral dose.

Anthelmintic activity was judged on the basis of total activity. By this criterion, Dose-Effect data for the compound gave the following results based on ova in fecal flotation and autopsy examination:

Dose Mg/Kg	No. dogs	No. positive post dose fecals		No. worms at autopsy	Percent dogs cleared
		#1	#2		
100	5	4/5	3/4*	0, 1, 15, 36, 50	20
125	6	2/5	0/3**	0, 0, 0, 0, 1, 1	67
150	10	2/10	1/10	0, 0, 0, 0, 0, 0, 0, 0, 5, 23	80
200	9	3/9	1/9	0, 0, 0, 0, 0, 0, 0, 0, 35	89

* 1 fecal not examined
** 3 fecals not examined

Lethal-Dose$_{50}$ trials, with doses of 750, 1000, 1500, and 3000 mg/kg, indicated that emesis functions as a protective mechanism. Thus, an LD$_{50}$ was not obtained by oral dose. Very few anthelmintics possess this type of activity

These data indicate a substance, unrelated to known anthelmintics, with interesting anthelmintic properties.—Frank A. Ehrenford, *Pitman-Moore Company, New Augusta, Indiana.*

SOME HELMINTHS PARASITIC IN *CLEMMYS MARMORATA*

During the period of May to October, 1953, 64 Pacific terrapins, *Clemmys marmorata* Baird and Girard, were dissected for helminth parasites. Fifty-two of the turtles had been trapped on the Rogue River drainage in Jackson County, Oregon, and 12 came from the Calapooya River in Linn County, which is a part of the Willamette River drainage, also in Oregon.

A total of 1,236 trematodes and nematodes were collected, comprising 7 species, of which 6 apparently represent new host records.

Two species of monogenetic trematode were encountered. About 32% of the turtles contained *Polystomoides coronatus* Leidy, and 3% were infected with *Neopolystoma orbiculare*

Stunkard. Among the digenetic flukes found was *Spirorchis artericola* Ward which was present in 14% of the turtles examined.

In addition, 12 specimens of a Paramphistomid fluke, tentatively identified as *Ophioxenos dienteros* Sumwalt, were taken from the cloacal area of 3 Calapooya River terrapins. This fluke was described from a garter snake, *Thamnophis* sp., and present material represents the first record for the species in a chelonian host.

The commonest digenetic fluke found in these turtles was the intestinal fluke *Telorchis corti* Stunkard. This species had been previously reported by Ingles (J. Parasit. 17: 101–103; 1930) from a Pacific terrapin in California. Present specimens showed an interesting age, or size, immunity to infection by this species. A greater incidence of infection, as well as larger infections, was encountered in the smaller turtles. Of the 16 terrapins from 10–13 cm. in carapace length 81% were parasitized with *T. corti* while the 31 specimens between 13 and 16 cm. showed only 10% of infection by this species. Four terrapins larger than 16 cm. were examined and none contained any specimens of this fluke.

Two species of nematode were encountered in *C. marmorata*. Some 31% of the turtles contained *Spironoura affine* Leidy, and 84% harbored *Spiroxys contortus* Rudolphi. Neither of these nematodes has previously been recorded from this host.—VERNON E. THATCHER, *Zoology Department, Oregon State College, Corvallis, Oregon.*

THE USE OF A SURFACE ACTIVE AGENT TO FACILITATE THE EXAMINATION OF INTESTINAL CONTENTS FOR HELMINTH PARASITES

During the course of quantitative studies on *Nippostrongylus muris* (Nematoda: Heligmosomidae) infections in laboratory rats, the accurate determination of worm burdens was hindered by the difficulty encountered in separating the parasites from mucous and other intestinal material. The usual procedure consisted of first scraping the mucosa and gut contents from a two to three inch segment of intestine into a counting plate. A small amount of water was added and the material was finely broken up with a bent glass rod and examined under a dissecting microscope.

The substitution of a nonionic surface active agent, Tween 80, for water greatly facilitated the procedure. The addition of 4 to 5 cc. of a 25 percent aqueous solution of the Tween 80 stock not only reduced the time required for breaking up the mucous and other intestinal material but also yielded a finer more easily examined preparation. The net result was that longer segments of intestine could be processed with greater accuracy in less time. The worms were not visibly injured by the treatment and remained viable throughout the period of counting and study.

There has been no opportunity to evaluate the method with other intestinal helminth infections and no comparative study with other surface active agents has been made. The present technic or modifications of it, however, should prove helpful in situations where a similar type of experimental design is employed.—A. JAMES HALEY, *Department of Parasitology, School of Hygiene and Public Health, The Johns Hopkins University, Baltimore, Md.*

FURTHER OBSERVATIONS ON *GLUGEA HERTWIGI* WEISSENBERG 1911, 1913 (MICROSPORIDIA) IN FRESH WATER SMELT IN NEW HAMPSHIRE

Because of its high value as a forage and food fish the restricted natural distribution of the fresh water smelt, *Osmerus mordax* (Mitchell), in New Hampshire has been greatly extended by transplantation. Each year during the spring spawning runs large quantities of smelt and smelt eggs are collected by the N.H. Fish and Game Department from Lake Winnisquam and distributed to other bodies of water throughout the state. During the late winter and spring of 1952, 910 smelt from Winnisquam were examined of which 86 (9.5 percent) were found infected with *Glugea hertwigi*. These data are particularly interesting when viewed according to the time of collection. Thus: February 9 (230 examined, none infected); April 22 (269 examined, 15 [5.6 percent] infected); May 9 to 19 (411 examined, 71 [17 percent] infected. Although the data appear to suggest a seasonal factor in the host-parasite relationship further study is needed to clarify this point.

O. mordax in N.H. is believed to be indigenous only in 2 or 3 bodies of water, one of which is Winnisquam, but through transplantation operations it has been introduced into nearly 100 other lakes and ponds. Since *G. hertwigi* was demonstrated in the transplanting stock at Winnisquam it was not surprising to find infected smelt populations elsewhere. Examination of nearly 500 smelt from several other localities, whose smelt populations were established from Winnisquam stock, showed the disease to be present in 7 additional lakes and ponds. Thus, to date the infection has been demonstrated in about 10 percent of the smelt populations in the state. The majority of localities, however, have not been studied and future surveys undoubtably will show diseased smelt in many of them.

Previous work by the author (Haley, J. Parasit. **38**: 183; 1952 and Trans Amer. Fish. Soc. In Press) and other investigators has demonstrated the severe pathological manifestation of the infection and in view of its extensive distribution and continued dissemination it is a problem of sufficient consequence to warrant serious consideration by individuals concerned with fisheries research in New Hampshire.—A. James Haley, *Department of Parasitology, School of Hygiene and Public Health, The Johns Hopkins University, Baltimore, Md.*

A NEW RECORD OF THE CESTODE *INFULA BURHINI* BURT (CYCLOPHYLLIDEA: DIOECOCESTIDAE) FROM AUSTRALIA

Among specimens kindly lent to us by Dr. E. W. Price, we found at least two fragmented cestodes (U. S. Nat. Mus. Helm. Coll. No. 33415) which are without doubt *Infula burhini* Burt, 1939 (Spolia Zeylanica, **21**: 195–208. The cestodes were collected by Dr. N. A. Cobb from the small intestine of a White-headed Stilt (*Himantopus himantopus leucocephalus*) in Australia. Precise locality data are not available. To our knowledge, *Infula burhini* has not been reported since its original description by Burt from *Burhinus oedicnemus* and *Himantopus himantopus* in Ceylon. Our observations on one male and one female specimen, agree in all particulars with those recorded by Burt (1939).—Marietta Voge, and Clark P. Read, *Departments of Infectious Diseases and Zoology, University of California, Los Angeles.*

SOME RECORDS OF ECTOPARASITIC ACARINA AND INSECTA FROM MAMMALS IN ONTARIO

In two communications previously published (Jour. Mammalogy, **31**: 357–358, 1950; **34**: 137–139, 1953) the writer reported upon collections of ectoparasitic ticks, mites and insects taken from mammals in southern Ontario. Since then a variety of specimens taken by several collectors from mammals in Ontario have been added to the collections of the University of Western Ontario. Most of the specimens have been mounted in Canada balsam on microscope slides, and in the following account the catalogue numbers of the slides are included. The name of the collector, where known, is also recorded. Each dated entry represents a collection from a single host. Identifications of the specimens were kindly made by the following taxonomists: G. F. Ferris, Natural History Museum, Stanford University, California (Anoplura), J. D. Gregson, Laboratory of Entomology, Department of Agriculture, Kamloops, B. C., (Ixodidae), G. P. Holland, Systematic Entomology, Department of Agriculture, Ottawa (Siphonaptera), H. H. J. Nesbitt, Department of Biology, Carleton College, Ottawa (Laelaptidae) and R. A. Ward, Department of Zoology, University of Chicago, Chicago, Illinois (Mallophaga).

ACARINA

Laelaptidae

Laelaps muris Ljungh—several mites from *Microtus p. pennsylvanicus* (Ord), Komoa, January 2, 1933, E. Davis (slide E16.49); one mite from *M. p. pennsylvanicus* (Ord), London Twp., Middlesex Co., February 4, 1952 (E16.62).

Laelaps microti (Ew.)—several mites from *Microtus p. pennsylvanicus* (Ord), Komoka, January 2, 1933, E. Davis (E16.49); several mites from *M. p. pennsylvanicus* (Ord), Melrose, December 21, 1940, N. R. Brown (E16.55).

Ixodidae

Ixodes cookei Packard—1 ♀ from *Mephitis mephitis* (Schreber), Byron, November 13, 1932, E. Davis (E16.48); 1 engorged ♀ from *Felis domesticus* L., a young kitten to which the tick was firmly attached in a pit in the skin between the right shoulder and the back, Delaware Twp., Middlesex Co., July 22, 1952, D. A. McLarty (E16.15); 1 ♀ from *Castor canadensis* Kuhl., Arden Lake, Arden Twp., Sudbury Dist., May 11, 1953, W. Punstel; 2 ♀♀ from *Marmota monax* (L.), a young animal of the year, London, June 18, 1953, W. W. Judd (E18.20, 21).

ANOPLURA

Haematopinidae

Hoplopleura acanthopus (Burm.)—8 lice from *Microtus p. pennsylvanicus* (Ord), London Twp., Middlesex Co., February 4, 1952 (E16.60, 61). G. F. Ferris (The sucking lice. Mem. Pacific Coast Entomol. Soc., vol. **1**, 1951) reports this species from several spp. of *Microtus*, not including *M. pennsylvanicus*.

Neohaematopinus sciurinus (Mjöberg)—3 lice from *Sciurus carolinensis leucotis* (Gapper), London, December 6, 1933, E. Davis (E16.36).

MALLOPHAGA

TRICHODECTIDAE

Eutrichophilus setosus Giebel—numerous ♂♂ and ♀♀ from *Erethizon dorsatum* (L.), Atikokan, June 2, 1933, E. Davis (E16.56–59). This sp. was recorded from *E. dorsatum* in Nova Scotia by G. B. Thompson (Can. Entomol., **66**: 279–281, 1934).

Trichodectes canis (DeGeer)—numerous ♂♂ and ♀♀ from *Canis* (*lupus* L.?), Maple, January 29, 1953 (E16.63–65). The host was a pup, taken from a den near Elizabethtown on February 26, 1952, and may have been an offspring from a mating of a wild wolf and a domestic dog. The parasites were removed from it on its death at Maple on January 29, 1953 after it had been kept in captivity since its original capture. G. B. Thompson (Can. Entomol., **66**: 279–281. 1934) records *T. canis* from a timber wolf at Hearst, Ont.

SIPHONAPTERA

HYSTRICHOPSYLLIDAE

Nearctopsylla g. genalis (Baker)—3 ♂♂, 1 ♀ from *Mustela erminea cicognanii* Bonaparte, December 28, 1932, Hough Lake, E. Davis (E16.40, 41).

Tamiophila grandis (Rothsch.)—1 ♀ from *Tamias striatus lysteri* (Richardson), September 2, 1932, Penetanguishene, E. Davis (E16.42).

Ceratophyllidae

Orchopeas caedens (subsp. *durus* ?) (Jordan)—1 ♀ from *Mustela vison* Schreber, March 20, 1953, Chaplin Twp., Sudbury Dist., T. Cachagee (E18.26). G. P. Holland (Siphonaptera of Canada. Dept. Agric., Tech. Bull. 80, 1949) lists this flea as a parasite of squirrels and includes some cases of its occurrence on *Mustela* spp. In the latter case the parasites presumably move to the predator from squirrels being devoured.

Orchopeas leucopus (Baker)—numerous ♂♂ and ♀♀ from *Peromyscus* sp. (captives in laboratory cages), May 19, 1951, London, R. H. Stinson (E17.92–100, E18.1–5).

COLEOPTERA

LEPTINIDAE

Leptinillus validus (Horn)—8 larvae from *Castor canadensis* Kuhl., January 21, 1954, Twp. 35, Chapleau Forestry Dist., V. Crichton (E18.22–25). The specimens were identified with keys in H. F. Chu (How to know the immature insects. W. C. Brown Co., Dubuque, Iowa, 1949). G. H. Horn described this species as *Leptinus validus* (Trans. Amer. Ent. Soc., **4**: 145–146, 1872) and discussed it further (Trans. Amer. Ent. Soc., **10**: 113–114, 1882), his specimens being from northern Ontario.—W. W. JUDD, *Dept. of Zoology, University of Western Ontario, London, Ontario.*

NOTES ON THE RABBIT TICK, *HAEMAPHYSALIS LEPORIS-PALUSTRIS* (PACKARD), AND TULAREMIA IN CENTRAL ALASKA

The first published reports on the distribution of the rabbit tick in Alaska resulted from investigations conducted during June and July of 1937. At that time it was found that "infestations of the rabbit tick, *Haemaphysalis leporis-palustris*, were remarkably localized." Although hares were examined at localities scattered all along the Steese and Richardson Highways and the Alaska Railroad, ticks were found only on hares from Seward and the immediate vicinity of College, northwest of Fairbanks (Philip, C. B.: J. Parasit. **24**: 483–488, 1938). *Bacterium tularense* was recovered from 3 samples of ticks from the College area (Philip, C. B. and R. R. Parker: Pub. Health Rep. **53**: 574–575, 1938).

No further studies were conducted until 1950, when a project was initiated by the Arctic Aeromedical Laboratory, Ladd Air Force Base, to investigate potential disease vectors in Alaska. During 1950 to 1952, *H. leporis-palustris* were collected from a total of 47 hares, but no *B. tularense* was found in these collections when tested by guinea pig injection. The ticks were distributed over a wider range than in 1937, at least as far as Circle Hot Springs to the northeast, Big Delta to the southeast, and the village of Clear to the southwest of Fairbanks.

The 1953 tick collections extended these earlier distribution records only to the west and northwest. Two ticks were removed from a hare found dead at Minto, and all of 19 hares examined at Livengood were infested. Despite the previous record from Circle Hot Springs, the ticks have apparently not become well established in the Central-Circle area, for all of the 29 hares examined from this area during 1953 were free from ticks.

Fourteen lots with a total of 430 *H. leporis-palustris* collected during 1953 were tested at

the Rocky Mountain Laboratory by injection of suspensions into guinea pigs. Aliquot portions of about half of these suspensions also were injected into baby mice to check for the presence of Colorado tick fever virus, but results of the latter tests were all negative.

Three of the above 14 tick lots yielded isolates of *B. tularense* in test guinea pigs (pure cultures were obtained after passage of each). Characteristic gross lesions also were observed at necropsy of these animals. The data on the ticks used in each of these 3 tests were as follows:

1. Lot 38: 24 adults and 3 nymphs taken from a pool of 271 ticks removed from 2 apparently healthy hares at College on May 19.
2. Lot 39: 5 females, 4 males, and 5 nymphs taken from a hare found dead in the College area by a University of Alaska student on May 21.
3. Lot 54: 1 engorged female taken from a sick hare found in the College area by a University of Alaska student on June 25.

Facilities for testing tissues from these two affected hare hosts unfortunately were not available at the time of discovery.

The presence of the low-grade type of Rocky Mountain spotted fever found in *H. leporis-palustris* studied previously at this Laboratory from other Nearctic areas, was suspected during the 1937 investigations in Alaska (Philip, C. B., 1938, l.c.). Similar findings also occurred in the tick tests during 1953. Two lots of ticks, which were treated with antibiotics to suppress contaminants, were injected directly into and passed in embryonated chicken eggs. Rickettsiae were observed in Giemsa-stained smears of the yolk sacs. Data on the tick lots were as follows:

1. Lot 37: 170 ticks (all stages) taken from 16 hares at Livengood on May 14.
2. Lot 38: 219 ticks (all stages) taken from the pool of 271 removed from two hares at College (different from the 27 ticks of this lot used for the tularemia tests reported above).

The egg-propagated rickettsial isolates, when injected into guinea pigs, were avirulent, caused no scrotal involvement, and could not be maintained in serial passage. However, 7 guinea pigs inoculated with these avirulent strains showed complete immunity on challenge with a fully virulent laboratory strain of Rock Mountain spotted fever, and the original infectious exposure of these animals followed by recovery was confirmed by positive complement fixation in sera from three animals before challenge.

From available data, it is evident that *H. leporis-palustris* has extended its known range in central Alaska, and it is possible that tularemia may show up in other areas through this medium. However, the very limited amount of research done on this problem in the Territory allows one to suspect that tularemia may already be present in other areas but simply has not been discovered. Rabbit ticks can be expected to fluctuate in abundance and distribution in accordance with well known hare cycles, and availability of hosts in a given range at different periods in such cycles. Two cases recorded by MacKinnon (J. Can. Med. Assoc. **56**: 541–542, 1947) from the Norman Wells region of the Mackenzie River Valley of Canada may indicate a wider distribution of tularemia in northern latitudes than is presently known.

Although a possible but unconfirmed case of tularemia from the vicinity of Wiseman was diagnosed in 1938 (Philip, C. B.: Proc. Sixth Pacific Sci. Cong. **5**: 71–73, 1939), the first serologically confirmed case of tularemia in Alaska following the handling of muskrats near Northway, was reported by Williams (Pub. Health Rep. **61**: 875–876, 1946). To date, 8 cases of tularemia from the Fairbanks-Steese Highway area have been diagnosed and confirmed (Pauls, F. P.: Significant Diseases in Alaska, Data Compiled for Indoctrination of Medical Officers at Elmendorf Air Force Base, Alaska, 1953). Incidental to our studies in 1951, serums from 3 old-time residents were tested for *B. tularense* agglutinins with negative results. These men had handled many hares while trapping and acting as big game guides between 20 and 30 years in several areas in Alaska.

Although this "increase" in human cases in recent years could be due to a greater awareness among local physicians of the presence of tularemia in this area, it is possible that the problem of tularemia is becoming more serious with increasing settlement and widening exposure. Since there are no ticks in Central Alaska that attack man, the chief source of human infection will continue to be the handling of snowshoe hares or other infected animals. Biting flies could be of incidental importance in the local presence of a hare epizootic, though tularemia is still not a known primary cause for widespread decimation of hares and rabbits (Philip, C. B., Bell, J. F., and Larson, C. L., 1953, XIV Intern. Zool. Cong. Copenhagen, in press). The problem affords an excellent opportunity for further research and it is expected to receive continuing attention by research personnel in Alaska.—Cornelius B. Philip, *Public Health Service, Rocky Mountain Laboratory, Hamilton, Montana;* Gordon D. Gill, 1st Lt., USAF (MSC), *Arctic Aeromedical Laboratory, Ladd Air Force Base, Alaska,* and John M. Geary, Maj., USAF (MSC), *now at Cornell University.*

EFFECTS OF DIETARY PROTEIN ON THE RELATIONSHIP BETWEEN LABORATORY MICE AND THE NEMATODE *NEMATOSPIROIDES DUBIUS*

Quantitative studies were conducted during 1950–52 on the effects of 10 and 20% dietary protein levels on the relationship between heterozygous albino female mice and *Nematospiroides dubius* (NEMATODA: HELIGMOSOMIDAE). These investigations were carried out under conditions of initial challenge of the host by *N. dubius*.

N. dubius used in these tests was recovered from *Peromyscus maniculatus gambelii* near Davis, Yolo County, California in 1950.

N. dubius was selected as the parasite because of several desirable characteristics: it has been shown to be pathogenic to mice by Spurlock (1943, J. Parasit. **29**: 303–311); infection occurs by ingestion; it produces a rapid host reaction; it is limited to gut wall tissue invasion; it persists in the host for about 8 months; and it is cultured with ease as a pure population in large numbers.

Mice in lots of 10 were tested for the effects of *N. dubius* on the growth curve as compared to uninfected controls: 100 larvae produced a slight but measurable temporary depression; 200 larvae produced a greater depression which was followed by an accelerated phase; 400 larvae caused 1 death in 20 days; 800 larvae caused 5 deaths in 20 days. A standard dose of 100 larvae per mouse was used in the subsequent tests.

It was found that 10 and 20% protein diets produced similar growth curves in mice, and that these curves were comparable to growth curves of mice on a commercial diet. No evidence was found by histopathological examination of protein depletion in mice on these two levels of dietary protein in 20 days. A diet containing less than 10% protein was found to be insufficient for maintenance.

Infected mice on the 20% protein level were protected against the effects of *N. dubius*, based upon growth curves and blood cellular changes. Infected mice on the 10% protein level suffered a temporary weight loss, and more severe blood changes than mice on the 20% protein diet. The effects of *N. dubius* on growth and the blood cellular elements were found to occur after the pre-patent period and at the onset of ovum production.

Diet appeared to have an appreciable effect on the survival and location of *N. dubius*. In older mice, 8.5% less worms survived on the 10% protein level than on the 20% protein; subsequent examination showed that these limited observations were not statistically significant. However, the 10% protein level restricted the distribution of the adult worms to a more anterior location than the 20% protein level.

An appreciable splenomegaly occurs in infected mice. Examination of liver and spleen sections revealed no discernible pathology attributable to *N. dubius*.

No evidence was found of secondary bacterial invasion in the gut lesions produced by *N. dubius*.—FRANK A. EHRENFORD, *Pitman-Moore Company, New Augusta, Indiana.*

News Note

THE SIXTH ANNUAL CONFERENCE OF MIDWESTERN PARASITOLOGISTS

The conference was held at Michigan State College June 21st and 22nd, 1954. There were 83 registered and people attending came from as far west as Kansas and south from Missouri and east from the Atlantic seaboard. A program of demonstrations totaling 18 varied presentations employed the first afternoon of the meeting. The banquet was held at the Kellogg Center where Dr. G. F. Otto was the speaker of the evening on the topic: "Mosquitoes, Worms, Samoans and the Parasitologist in Samoa." The address was punctuated with a fine series of Kodachrome slides. The second day of the meeting consisted of a business meeting in which Dr. R. M. Cable was elected presiding officer for the coming year. After the election everyone enjoyed a graduate student panel discussion of problems in the training of advanced students in parasitology.

The committees on next year's meeting place and program were not able to come to a decision so this will be announced later in the year.

This organization, although primarily in the midwest, welcomes any interested persons to attend and participate in the meetings.—William D. Lindquist, Sec.-Treas.

Dept. of Bacteriology
Michigan State College
East Lansing, Michigan.

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